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(54) Title: METHODS AND COMPOSITIONS FOR EDITING NUCLEOTIDE SEQUENCES

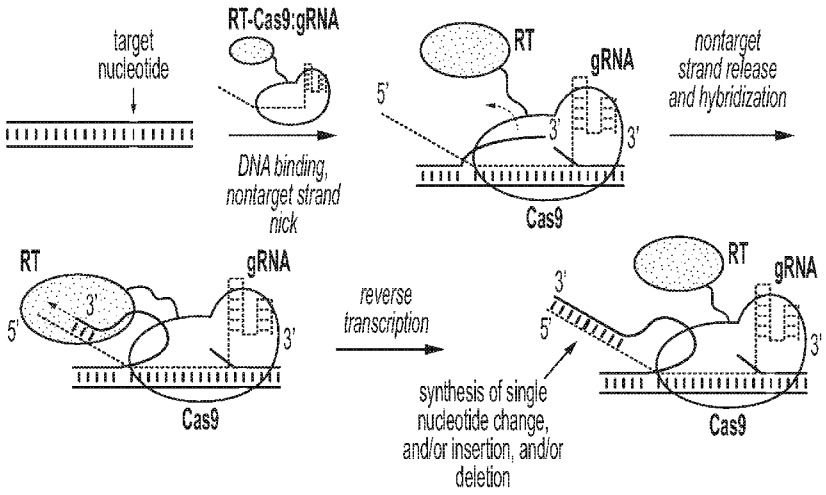

FIG. 1A

(57) Abstract: The present disclosure provides compositions and methods for conducting prime editing of a target DNA molecule (e.g., a genome) that enables the incorporation of a nucleotide change and/or targeted mutagenesis. The nucleotide change can include a single-nucleotide change (e.g., any transition or any transversion), an insertion of one or more nucleotides, or a deletion of one or more nucleotides. More in particular, the disclosure provides fusion proteins comprising nucleic acid programmable DNA binding proteins (napDNAbp) and a polymerase (e.g., reverse transcriptase), which is guided to a specific DNA sequence by a modified guide RNA, named an PEgRNA. The PEgRNA has been altered (relative to a standard guide RNA) to comprise an extended portion that provides a DNA synthesis template sequence which encodes a single strand DNA flap, which is homologous to a strand of the targeted endogenous DNA sequence to be edited, but which contains the desired one or more nucleotide changes and which, following synthesis

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[Continued on next page]

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by the polymerase (e.g., reverse transcriptase), becomes incoporated into the target DNA molecule. Also disclosed herein are various methods that leverage prime editing, including treating trinucleotide repeat contraction diseases, installing targeted peptide tags, treating prion disease through the intallation of protection mutations, manipulating RNA-encoding genes for the installation of RNA tags for controlling the function and expression of RNA, using prime editing to construct sophisticated gene libraries, using prime editing to insert immunoepitopes into proteins, use of prime editing to insert inducible dimerization domains into protein targets, and delivery methods, among others.

METHODS AND COMPOSITIONS FOR EDITING NUCLEOTIDE SEQUENCES

GOVERNMENT SUPPORT

[0001] This invention was made with government support under grant numbers U01AI142756, RM1HG009490, R01EB022376, and R35GM118062 awarded by the National Institutes of Health. The government has certain rights in the invention.

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0002] This U.S. Provisional Application refers to and incorporates by reference the following applications, namely, U.S. Provisional Application No. 62/820,813, filed March 19, 2019 (Attorney Docket No. B1195.70074US00), U.S. Provisional Application No. 62/858,958 (Attorney Docket No. B1195.70074US01), filed June 7, 2019, U.S. Provisional Application No. 62/889,996 (Attorney Docket No. B1195.70074US02), filed August 21, 2019, U.S. Provisional Application No. 62/922,654, filed August 21, 2019 (Attorney Docket No. B1195.70083US00), U.S. Provisional Application No. 62/913,553 (Attorney Docket No. B1195.70074US03), filed October 10, 2019, U.S. Provisional Application No. 62/973,558 (Attorney Docket No. B1195.70083US01), filed October 10, 2019, U.S. Provisional Application No. 62/931,195 (Attorney Docket No. B1195.70074US04), filed November 5, 2019, U.S. Provisional Application No. 62/944,231 (Attorney Docket No. B1195.70074US05), filed December 5, 2019, U.S. Provisional Application No. 62/974,537 (Attorney Docket No. B1195.70083US02), filed December 5, 2019, U.S. Provisional Application No. 62/991,069 (Attorney Docket No. B1195.70074US06), filed March 17, 2020, and U.S. Provisional Application No. (serial number not available as of this filing) (Attorney Docket No. B1195.70083US03), filed March 17, 2020.

BACKGROUND OF THE INVENTION

[0003] Pathogenic single nucleotide mutations contribute to approximately 50% of human diseases for which there is a genetic component,[7] according to some estimates. Unfortunately, treatment options for patients with these genetic disorders remain extremely limited, despite decades of gene therapy exploration[8]. Perhaps the most parsimonious solution to this therapeutic challenge is direct correction of single nucleotide mutations in patient genomes, which would address the root cause of disease and would likely provide lasting benefit. Although such a strategy was previously unthinkable, recent improvements in genome editing capabilities brought about by the advent of the CRISPR/Cas system[9] have

now brought this therapeutic approach within reach. By straightforward design of a guide RNA (gRNA) sequence that contains ~20 nucleotides complementary to the target DNA sequence, nearly any conceivable genomic site can be specifically accessed by CRISPR associated (Cas) nucleases[1,2]. To date, several monomeric bacterial Cas nuclease systems have been identified and adapted for genome editing applications[10]. This natural diversity of Cas nucleases, along with a growing collection of engineered variants[11–14], offers fertile ground for developing new genome editing technologies.

[0004] While gene disruption with CRISPR is now a mature technique, precision editing of single base pairs in the human genome remains a major challenge[3]. Homology directed repair (HDR) has long been used in human cells and other organisms to insert, correct, or exchange DNA sequences at sites of double strand breaks (DSBs) using donor DNA repair templates that encode the desired edits[15]. However, traditional HDR has very low efficiency in most human cell types, particularly in non-dividing cells, and competing non-homologous end joining (NHEJ) leads predominantly to insertion-deletion (indel) byproducts[16]. Other issues relate to the generation of DSBs, which can give rise to large chromosomal rearrangements and deletions at target loci[17], or activate the p53 axis leading to growth arrest and apoptosis[18,19].

[0005] Several approaches have been explored to address these drawbacks of HDR. For example, repair of single-stranded DNA breaks (nicks) with oligonucleotide donors has been shown to reduce indel formation, but yields of desired repair products remain low[20]. Other strategies attempt to bias repair toward HDR over NHEJ using small molecule and biologic reagents[21–23]. However, the effectiveness of these methods is likely cell-type dependent, and perturbation of the normal cell state could lead to undesirable and unforeseeable effects.

[0006] Recently, the inventors, led by Prof. David Liu et al., developed base editing as a technology that edits target nucleotides without creating DSBs or relying on HDR[4–6,24–27]. Direct modification of DNA bases by Cas-fused deaminase enzymes allows for C•G to T•A, or A•T to G•C, base pair conversions in a short target window (~5-7 bases) with very high efficiency. As a result, base editors have been rapidly adopted by the scientific community. However, the following factors limit their generality for precision genome editing: (1) "bystander editing" of non-target C or A bases within the target window are observed; (2) target nucleotide product mixtures are observed; (3) target bases must be located 15±2 nucleotides upstream of a PAM sequence; and (5) repair of small insertion and deletion mutations is not possible.

[0007] Therefore, the development of programmable editors that are flexibly capable of introducing any desired single nucleotide change and/or which could install base pair insertions or deletions (e.g., at least 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more base pair insertions or deletions) and/or which could alter or modify the nucleotide sequence at a target site with high specificity and efficiency would substantially expand the scope and therapeutic potential of genome editing technologies based on CRISPR.

SUMMARY OF THE INVENTION

[0008] The present invention describes an entirely new platform for genome editing called "prime editing." Prime editing is a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a nucleic acid programmable DNA binding protein ("napDNAbp") working in association with a polymerase (i.e., in the form of a fusion protein or otherwise provided *in trans* with the napDNAbp), wherein the prime editing system is programmed with a prime editing (PE) guide RNA ("PEgRNA") that both specifies the target site and templates the synthesis of the desired edit in the form of a replacement DNA strand by way of an extension (either DNA or RNA) engineered onto a guide RNA (e.g., at the 5′ or 3′ end, or at an internal portion of a guide RNA). The replacement strand containing the desired edit (e.g., a single nucleobase substitution) shares the same sequence as the endogenous strand of the target site to be edited (with the exception that it includes the desired edit). Through DNA repair and/or replication machinery, the endogenous strand of the target site is replaced by the newly synthesized replacement strand containing the desired edit. In some cases, prime editing may be thought of as a "search-and-replace" genome editing technology since the prime editors, as described herein, not only search and locate the desired target site to be edited, but at the same time, encode a replacement strand containing a desired edit which is installed in place of the corresponding target site endogenous DNA strand.

[0009] The prime editors of the present disclosure relate, in part, to the discovery that the mechanism of target-primed reverse transcription (TPRT) or "prime editing" can be leveraged or adapted for conducting precision CRISPR/Cas-based genome editing with high efficiency and genetic flexibility (e.g., as depicted in various embodiments of FIGs. 1A-1F). TPRT is naturally used by mobile DNA elements, such as mammalian non-LTR retrotransposons and bacterial Group II introns[28,29]. The inventors have herein used Cas protein-reverse transcriptase fusions or related systems to target a specific DNA sequence

with a guide RNA, generate a single strand nick at the target site, and use the nicked DNA as a primer for reverse transcription of an engineered reverse transcriptase template that is integrated with the guide RNA. However, while the concept begins with prime editors that use reverse transcriptases as the DNA polymerase component, the prime editors described herein are not limited to reverse transcriptases but may include the use of virtually and DNA polymerase. Indeed, while the application throughout may refer to prime editors with "reverse transcriptases," it is set forth here that reverse transcriptases are only one type of DNA polymerase that may work with prime editing. Thus, where ever the specification mentions "reverse transcriptases," the person having ordinary skill in the art should appreciate that any suitable DNA polymerase may be used in place of the reverse transcriptase. Thus, in one aspect, the prime editors may comprise Cas9 (or an equivalent napDNAbp) which is programmed to target a DNA sequence by associating it with a specialized guide RNA (i.e., PEgRNA) containing a spacer sequence that anneals to a complementary protospacer in the target DNA. The specialized guide RNA also contains new genetic information in the form of an extension that encodes a replacement strand of DNA containing a desired genetic alteration which is used to replace a corresponding endogenous DNA strand at the target site. To transfer information from the PEgRNA to the target DNA, the mechanism of prime editing involves nicking the target site in one strand of the DNA to expose a 3′-hydroxyl group. The exposed 3′-hydroxyl group can then be used to prime the DNA polymerization of the edit-encoding extension on PEgRNA directly into the target site. In various embodiments, the extension—which provides the template for polymerization of the replacement strand containing the edit—can be formed from RNA or DNA. In the case of an RNA extension, the polymerase of the prime editor can be an RNA-dependent DNA polymerase (such as, a reverse transcriptase). In the case of a DNA extension, the polymerase of the prime editor may be a DNA-dependent DNA polymerase.

[0010] The newly synthesized strand (i.e., the replacement DNA strand containing the desired edit) that is formed by the herein disclosed prime editors would be homologous to the genomic target sequence (i.e., have the same sequence as) except for the inclusion of a desired nucleotide change (e.g., a single nucleotide change, a deletion, or an insertion, or a combination thereof). The newly synthesized (or replacement) strand of DNA may also be referred to as a single strand DNA flap, which would compete for hybridization with the complementary homologous endogenous DNA strand, thereby displacing the corresponding endogenous strand. In certain embodiments, the system can be combined with the use of an

error-prone reverse transcriptase enzyme (e.g., provided as a fusion protein with the Cas9 domain, or provided *in trans* to the Cas9 domain). The error-prone reverse transcriptase enzyme can introduce alterations during synthesis of the single strand DNA flap. Thus, in certain embodiments, error-prone reverse transcriptase can be utilized to introduce nucleotide changes to the target DNA. Depending on the error-prone reverse transcriptase that is used with the system, the changes can be random or non-random.

[0011] Resolution of the hybridized intermediate (comprising the single strand DNA flap synthesized by the reverse transcriptase hybridized to the endogenous DNA strand) can include removal of the resulting displaced flap of endogenous DNA (e.g., with a 5′ end DNA flap endonuclease, FEN1), ligation of the synthesized single strand DNA flap to the target DNA, and assimilation of the desired nucleotide change as a result of cellular DNA repair and/or replication processes. Because templated DNA synthesis offers single nucleotide precision for the modification of any nucleotide, including insertions and deletions, the scope of this approach is very broad and could foreseeably be used for myriad applications in basic science and therapeutics.

[0012] In one aspect, the specification provides a fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase. In various embodiments, the fusion protein is capable of carrying out genome editing by target-primed reverse transcription in the presence of an extended guide RNA.

[0013] In certain embodiments, the napDNAbp has a nickase activity. The napDNAbp may also be a Cas9 protein or functional equivalent thereof, such as a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[0014] In certain embodiments, the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[0015] In other embodiments, the fusion protein when complexed with an extended guide RNA is capable of binding to a target DNA sequence.

[0016] In still other embodiments, the target DNA sequence comprises a target strand and a complementary non-target strand.

[0017] In other embodiments, the binding of the fusion protein complexed to the extended guide RNA forms an R-loop. The R-loop can comprise (i) an RNA-DNA hybrid comprising the extended guide RNA and the target strand, and (ii) the complementary non-target strand.

[0018] In still other embodiments, the complementary non-target strand is nicked to form a reverse transcriptase priming sequence having a free 3′ end.

[0019] In various embodiments, the extended guide RNA comprises (a) a guide RNA and (b) an RNA extension at the 5′ or the 3′ end of the guide RNA, or at an intramolecular location in the guide RNA. The RNA extension can comprise (i) a reverse transcription template sequence comprising a desired nucleotide change, (ii) a reverse transcription primer binding site, and (iii) optionally, a linker sequence. In various embodiments, the reverse transcription template sequence may encode a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

[0020] In various embodiments, the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[0021] In still other embodiments, the single-strand DNA flap may hybridize to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change. In still other embodiments, the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5′ end. In certain embodiments, the displaced endogenous DNA having the 5′ end is excised by the cell.

[0022] In various embodiments, the cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[0023] In various other embodiments, the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence.

[0024] In still other embodiments, the desired nucleotide change is installed in an editing window that is between about -5 to +5 of the nick site, or between about -10 to +10 of the nick site, or between about -20 to +20 of the nick site, or between about -30 to +30 of the nick site, or between about -40 to + 40 of the nick site, or between about -50 to +50 of the nick site, or between about -60 to +60 of the nick site, or between about -70 to +70 of the nick site, or between about -80 to +80 of the nick site, or between about -90 to +90 of the

nick site, or between about -100 to +100 of the nick site, or between about -200 to +200 of the nick site.

[0025] In various embodiments, the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18. In various other embodiments, the napDNAbp comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 26-39, 42- 61, 75-76, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487 (Cas9); (SpCas9); SEQ ID NO: 77-86 (CP-Cas9); SEQ ID NO: 18-25 and 87-88 (SpCas9); and SEQ ID NOs: 62-72(Cas12)

[0026] In other embodiments, the reverse transcriptase of the disclosed fusion proteins and/or compositions may comprise any one of the amino acid sequences of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700-716, 739-742, and 766. In still other embodiments, the reverse transcriptase may comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700-716, 739-742, and 766. These sequences may be naturally occurring reverse transcriptase sequences, e.g., from a retrovirus or a retrotransposon, of the sequences may be recombinant.

[0027] In various other embodiments, the fusion proteins herein disclosed may comprise various structural configurations. For example, the fusion proteins may comprise the structure NH_2-[napDNAbp]-[reverse transcriptase]-COOH; or NH_2-[reverse transcriptase]-[napDNAbp]-COOH, wherein each instance of "]-["indicates the presence of an optional linker sequence.

[0028] In various embodiments, the linker sequence comprises an amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769, or an amino acid sequence that this at least 80%, 85%, or 90%, or 95%, or 99% identical to any one of the linker amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[0029] In various embodiments, the desired nucleotide change that is incorporated into the target DNA can be a single nucleotide change (e.g., a transition or transversion), an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

[0030] In certain cases, the insertion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 30, at least

40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, or at least 500 nucleotides in length.

[0031] In certain other cases, the deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, or at least 500 nucleotides in length.

[0032] In another aspect, the present disclosure provides an extended guide RNA comprising a guide RNA and at least one RNA extension. The RNA extension can be positioned at the 3′ end of the guide RNA. In other embodiments, the RNA extension can be positioned at the 5′ of the guide RNA. In still other embodiments, the RNA extension can be positioned at an intramolecular position within the guide RNA, however, preferable, the intramolecular positioning of the extended portion does not disrupt the functioning of the protospacer.

[0033] In various embodiments, the extended guide RNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence. The target DNA sequence can comprise a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[0034] In various embodiments of the extended guide RNA, the at least one RNA extension can comprise a reverse transcription template sequence. In various other embodiment, the RNA extension may further comprises a reverse transcription primer binding site. In still further embodiments, the RNA extension may comprise a linker or spacer sequence that joins the RNA extension to the guide RNA.

[0035] In various embodiments, the RNA extension can be at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 150 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0036] In other embodiments, the reverse transcription template sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0037] In still other embodiments, wherein the reverse transcription primer binding site sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0038] In other embodiments, the optional linker or spacer sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0039] In various embodiments of the extended guide RNAs, the reverse transcription template sequence may encode a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change. The single-stranded DNA flap may displace an endogenous single-strand DNA at the nick site. The displaced endogenous single-strand DNA at the nick site can have a 5´ end and form an endogenous flap, which can be excised

by the cell. In various embodiments, excision of the 5′ end endogenous flap can help drive product formation since removing the 5′ end endogenous flap encourages hybridization of the single-strand 3′ DNA flap to the corresponding complementary DNA strand, and the incorporation or assimilation of the desired nucleotide change carried by the single-strand 3′ DNA flap into the target DNA.

[0040] In various embodiments of the extended guide RNAs, the cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[0041] [0001] In certain embodiments, the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 131 , 222 , 394, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 641, 642, 643, 644, 645, 646, 647, 648, 649, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 738, 2997, 2998, 2999, 3000, 3001, 3002, 3003, 3004, 3005, 3006, 3007, 3008, 3009, 3010, 3011, 3012, 3013, 3014, 3015, 3016, 3017, 3018, 3019, 3020, 3021, 3022, 3023, 3024, 3025, 3026, 3027, 3028, 3029, 3030, 3031, 3032, 3033, 3034, 3035, 3036, 3037, 3038, 3039, 3040, 3041, 3042, 3043, 3044, 3045, 3046, 3047, 3048, 3049, 3050, 3051, 3052, 3053, 3054, 3055, 3056, 3057, 3058, 3059, 3060, 3061, 3062, 3063, 3064, 3065, 3066, 3067, 3068, 3069, 3070, 3071, 3072, 3073, 3074, 3075, 3076, 3077, 3078, 3079, 3080, 3081, 3082, 3083, 3084, 3085, 3086, 3087, 3088, 3089, 3090, 3091, 3092, 3093, 3094, 3095, 3096, 3097, 3098, 3099, 3100, 3101, 3102, 3103, 3113, 3114, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3305, 3306, 3307, 3308, 3309, 3310, 3311, 3312, 3313, 3314, 3315, 3316, 3317, 3318, 3319, 3320, 3321, 3322, 3323, 3324, 3325, 3326, 3327, 3328, 3329, 3330, 3331, 3332, 3333, 3334, 3335, 3336, 3337, 3338, 3339, 3340, 3341, 3342, 3343, 3344, 3345, 3346, 3347, 3348, 3349, 3350, 3351, 3352, 3353, 3354, 3355, 3356, 3357, 3358, 3359, 3360, 3361, 3362, 3363, 3364, 3365, 3366, 3367, 3368, 3369, 3370, 3371, 3372, 3373, 3374, 3375, 3376, 3377, 3378, 3379, 3380, 3381, 3382, 3383, 3384, 3385, 3386, 3387, 3388, 3389, 3390, 3391, 3392, 3393, 3394, 3395, 3396, 3397, 3398, 3399, 3400, 3401, 3402, 3403, 3404, 3405, 3406, 3407, 3408, 3409, 3410, 3411, 3412, 3413, 3414, 3415, 3416, 3417, 3418, 3419, 3420, 3421, 3422, 3423, 3424, 3425, 3426, 3427, 3428, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3437, 3438, 3439, 3440, 3441, 3442, 3443, 3444, 3445, 3446, 3447, 3448, 3449, 3450, 3451, 3452, 3453, 3454, 3455, 3479, 3480, 3481, 3482, 3483, 3484, 3485, 3486, 3487, 3488, 3489, 3490, 3491, 3492, 3493, 3522, 3523, 3524, 3525, 3526, 3527, 3528, 3529, 3530, 3531, 3532, 3533, 3534, 3535, 3536, 3537, 3538, 3539, 3540, 3549, 3550, 3551, 3552, 3553, 3554, 3555, 3556, 3628, 3629, 3630, 3631, 3632, 3633, 3634, 3635, 3636, 3637, 3638, 3639, 3640,

3641, 3642, 3643, 3644, 3645, 3646, 3647, 3648, 3649, 3650, 3651, 3652, 3653, 3654, 3655, 3656, 3657, 3658, 3659, 3660, 3661, 3662, 3663, 3664, 3665, 3666, 3667, 3668, 3669, 3670, 3671, 3672, 3673, 3674, 3675, 3676, 3677, 3678, 3679, 3680, 3681, 3682, 3683, 3684, 3685, 3686, 3687, 3688, 3689, 3690, 3691, 3692, 3693, 3694, 3695, 3696, 3697, 3698, 3755, 3756, 3757, 3758, 3759, 3760, 3761, 3762, 3763, 3764, 3765, 3766, 3767, 3768, 3769, 3770, 3771, 3772, 3773, 3774, 3775, 3776, 3777, 3778, 3779, 3780, 3781, 3782, 3783, 3784, 3785, 3786, 3787, 3788, 3789, 3790, 3791, 3792, 3793, 3794, 3795, 3796, 3797, 3798, 3799, 3800, 3801, 3802, 3803, 3804, 3805, 3806, 3807, 3808, 3809, and 3810 , or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 131 , 222 , 394, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 641, 642, 643, 644, 645, 646, 647, 648, 649, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 738, 2997, 2998, 2999, 3000, 3001, 3002, 3003, 3004, 3005, 3006, 3007, 3008, 3009, 3010, 3011, 3012, 3013, 3014, 3015, 3016, 3017, 3018, 3019, 3020, 3021, 3022, 3023, 3024, 3025, 3026, 3027, 3028, 3029, 3030, 3031, 3032, 3033, 3034, 3035, 3036, 3037, 3038, 3039, 3040, 3041, 3042, 3043, 3044, 3045, 3046, 3047, 3048, 3049, 3050, 3051, 3052, 3053, 3054, 3055, 3056, 3057, 3058, 3059, 3060, 3061, 3062, 3063, 3064, 3065, 3066, 3067, 3068, 3069, 3070, 3071, 3072, 3073, 3074, 3075, 3076, 3077, 3078, 3079, 3080, 3081, 3082, 3083, 3084, 3085, 3086, 3087, 3088, 3089, 3090, 3091, 3092, 3093, 3094, 3095, 3096, 3097, 3098, 3099, 3100, 3101, 3102, 3103, 3113, 3114, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3305, 3306, 3307, 3308, 3309, 3310, 3311, 3312, 3313, 3314, 3315, 3316, 3317, 3318, 3319, 3320, 3321, 3322, 3323, 3324, 3325, 3326, 3327, 3328, 3329, 3330, 3331, 3332, 3333, 3334, 3335, 3336, 3337, 3338, 3339, 3340, 3341, 3342, 3343, 3344, 3345, 3346, 3347, 3348, 3349, 3350, 3351, 3352, 3353, 3354, 3355, 3356, 3357, 3358, 3359, 3360, 3361, 3362, 3363, 3364, 3365, 3366, 3367, 3368, 3369, 3370, 3371, 3372, 3373, 3374, 3375, 3376, 3377, 3378, 3379, 3380, 3381, 3382, 3383, 3384, 3385, 3386, 3387, 3388, 3389, 3390, 3391, 3392, 3393, 3394, 3395, 3396, 3397, 3398, 3399, 3400, 3401, 3402, 3403, 3404, 3405, 3406, 3407, 3408, 3409, 3410, 3411, 3412, 3413, 3414, 3415, 3416, 3417, 3418, 3419, 3420, 3421, 3422, 3423, 3424, 3425, 3426, 3427, 3428, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3437, 3438, 3439, 3440, 3441, 3442, 3443, 3444, 3445, 3446, 3447, 3448, 3449, 3450, 3451, 3452, 3453, 3454, 3455, 3479, 3480, 3481, 3482, 3483, 3484, 3485, 3486, 3487, 3488, 3489, 3490, 3491, 3492, 3493, 3522, 3523, 3524, 3525, 3526, 3527, 3528, 3529, 3530, 3531, 3532, 3533, 3534, 3535, 3536, 3537, 3538, 3539, 3540, 3549, 3550, 3551, 3552, 3553, 3554, 3555, 3556, 3628, 3629, 3630, 3631, 3632, 3633, 3634, 3635, 3636,

3637, 3638, 3639, 3640, 3641, 3642, 3643, 3644, 3645, 3646, 3647, 3648, 3649, 3650, 3651, 3652, 3653, 3654, 3655, 3656, 3657, 3658, 3659, 3660, 3661, 3662, 3663, 3664, 3665, 3666, 3667, 3668, 3669, 3670, 3671, 3672, 3673, 3674, 3675, 3676, 3677, 3678, 3679, 3680, 3681, 3682, 3683, 3684, 3685, 3686, 3687, 3688, 3689, 3690, 3691, 3692, 3693, 3694, 3695, 3696, 3697, 3698, 3755, 3756, 3757, 3758, 3759, 3760, 3761, 3762, 3763, 3764, 3765, 3766, 3767, 3768, 3769, 3770, 3771, 3772, 3773, 3774, 3775, 3776, 3777, 3778, 3779, 3780, 3781, 3782, 3783, 3784, 3785, 3786, 3787, 3788, 3789, 3790, 3791, 3792, 3793, 3794, 3795, 3796, 3797, 3798, 3799, 3800, 3801, 3802, 3803, 3804, 3805, 3806, 3807, 3808, 3809, and 3810.

[0042] In yet another aspect of the invention, the specification provides for complexes comprising a fusion protein described herein and any extended guide RNA described above.

[0043] In still other aspects of the invention, the specification provides a complex comprising a napDNAbp and an extended guide RNA. The napDNAbp can be a Cas9 nickase, or can be an amino acid sequence of SEQ ID NOs: 42-57 (Cas9 nickase) and 65 (AsCas12a nickase), or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 42-57 (Cas9 nickase) and 65 (AsCas12a nickase).

[0044] In various embodiments involving a complex, the extended guide RNA is capable of directing the napDNAbp to a target DNA sequence. In various embodiments, a reverse transcriptase may be provided *in trans*, i.e., provided from a different source than the complex itself. For example, a reverse transcriptase could be provided to the same cell having the complex by introducing a separate vector separately encoding the reverse transcriptase.

[0045] In yet another aspect, the specification provides polynucleotides. In certain embodiments, the polynucleotides may encode any of the fusion proteins disclosed herein. In certain other embodiments, the polynucleotides may encode any of the napDNAbps disclosed herein. In still further embodiments, the polynucleotides may encode any of the reverse transcriptases disclosed herein. In yet other embodiments, the polynucleotides may encode any of the extended guide RNAs disclosed herein, any of the reverse transcription template sequences, or any of the reverse transcription primer sites, or any of the optional linker sequences.

[0046] In still other aspects, the specification provides vectors comprising the polynucleotides described herein. Thus, in certain embodiments, the vectors comprise polynucleotides for encoding the fusion proteins comprising a napDNAbp and a reverse

transcriptase. In other embodiments, the vectors comprise polynucleotides that separately encode a napDNAbp and reverse transcriptase. In still other embodiments, the vectors may comprise polynucleotides that encode the extended guide RNAs. In various embodiments, the vectors may comprise one or more polynucleotides that encode napDNAbps, reverse transcriptase, and extended guide RNAs on the same or separate vectors.

[0047] In still other aspects, the specification provides cells comprising a fusion protein as described herein and an extended guide RNA. The cells may be transformed with the vectors comprising the fusion proteins, napDNAbps, reverse transcriptase, and extended guide RNAs. These genetic elements may be comprised on the same vectors or on different vectors.

[0048] In yet another aspect, the specification provides pharmaceutical compositions. In certain embodiments, the pharmaceutical compositions comprise one or more of a napDNAbp, a fusion protein, a reverse transcriptase, and an extended guide RNA. In certain embodiments, the fusion protein described herein and a pharmaceutically acceptable excipient. In other embodiments, the pharmaceutical compositions comprise any extend guide RNA described herein and a pharmaceutically acceptable excipient. In still other embodiments, the pharmaceutical compositions comprise any extend guide RNA described herein in combination with any fusion protein described herein and a pharmaceutically acceptable excipient. In yet other embodiments, the pharmaceutical compositions comprise any polynucleotide sequence encoding one or more of a napDNAbp, a fusion protein, a reverse transcriptase, and an extended guide RNA. In still other embodiments, the various components disclosed herein may be separated into one or more pharmaceutical compositions. For example, a first pharmaceutical composition may comprise a fusion protein or a napDNAbp, a second pharmaceutical compositions may comprise a reverse transcriptase, and a third pharmaceutical composition may comprise an extended guide RNA.

[0049] In still a further aspect, the present disclosure provides kits. In one embodiment, the kit comprises one or more polynucleotides encoding one or more components, including a fusion protein, a napDNAbp, a reverse transcriptase, and an extended guide RNA. The kits may also comprise vectors, cells, and isolated preparations of polypeptides, including any fusion protein, napDNAbp, or reverse transcriptase disclosed herein.

[0050] In yet another aspect, the present disclosure provides for methods of using the disclosed compositions of matter.

[0051] In one embodiment, the methods relate to a method for installing a desired nucleotide change in a double-stranded DNA sequence. The method first comprises contacting the double-stranded DNA sequence with a complex comprising a fusion protein and an extended guide RNA, wherein the fusion protein comprises a napDNAbp and a reverse transcriptase and wherein the extended guide RNA comprises a reverse transcription template sequence comprising the desired nucleotide change. Next, the method involves nicking the double-stranded DNA sequence on the non-target strand, thereby generating a free single-strand DNA having a 3´ end. The method then involves hybridizing the 3` end of the free single-strand DNA to the reverse transcription template sequence, thereby priming the reverse transcriptase domain. The method then involves polymerizing a strand of DNA from the 3´ end, thereby generating a single-strand DNA flap comprising the desired nucleotide change. Then, the method involves replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

[0052] In other embodiments, the disclosure provides for a method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a guide RNA which targets the napDNAbp to the target locus, wherein the guide RNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change. Next, the method involves forming an exposed 3´ end in a DNA strand at the target locus and then hybridizing the exposed 3´ end to the RT template sequence to prime reverse transcription. Next, a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence is synthesized or polymerized by reverse transcriptase. Lastly, the at least one desired nucleotide change is incorporated into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

[0053] In still other embodiments, the disclosure provides a method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired

nucleotide change; and (c) incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

[0054] In certain embodiments, the step of replacing the endogenous DNA strand comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

[0055] In various embodiments, the desired nucleotide change can be a single nucleotide substitution (e.g., and transition or a transversion change), a deletion, or an insertion. For example, the desired nucleotide change can be (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[0056] In other embodiments, the desired nucleoid change can convert (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[0057] In still other embodiments, the method introduces a desired nucleotide change that is an insertion. In certain cases, the insertion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, or at least 500 nucleotides in length.

[0058] In other embodiments, the method introduces a desired nucleotide change that is a deletion. In certain other cases, the deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, or at least 500 nucleotides in length.

[0059] In various embodiments, the desired nucleotide change corrects a disease-associated gene. The disease-associated gene can be associated with a monogenetic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease. In other embodiments, the disease-associated gene can be associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[0060] The methods disclosed herein may involve fusion proteins having a napDNAbp that is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9. In other embodiments, a napDNAbp and reverse transcriptase are not encoded as a single fusion protein, but rather can be provided in separate constructs. Thus, in some embodiments, the reverse transcriptase can be provided *in trans* relative to the napDNAbp (rather than by way of a fusion protein).

[0061] In various embodiments involving methods, the napDNAbp may comprise an amino acid sequence of SEQ ID NOs: 26- 61, 75-76, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487 (Cas9); (SpCas9); SEQ ID NO: 77-86 (CP-Cas9); SEQ ID NO: 18 -25 and 87-88 (SpCas9); and SEQ ID NOs: 62-72(Cas12). The napDNAbp may also comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 26- 61, 75-76, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487 (Cas9); (SpCas9); SEQ ID NO: 77-86 (CP-Cas9); SEQ ID NO: 18 -25 and 87-88 (SpCas9); and SEQ ID NOs: 62-72(Cas12).

[0062] In various embodiments involving methods, the reverse transcriptase may comprise any one of the amino acid sequences of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700-716, 739-742, and 766. The reverse transcriptase may also comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700-716, 739-742, and 766.

[0063] The methods may involve the use of a PEgRNA comprising a nucleotide sequence of SEQ ID NOs: 131, 222, 394, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441,

442, 641, 642, 643, 644, 645, 646, 647, 648, 649, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 738, 2997, 2998, 2999, 3000, 3001, 3002, 3003, 3004, 3005, 3006, 3007, 3008, 3009, 3010, 3011, 3012, 3013, 3014, 3015, 3016, 3017, 3018, 3019, 3020, 3021, 3022, 3023, 3024, 3025, 3026, 3027, 3028, 3029, 3030, 3031, 3032, 3033, 3034, 3035, 3036, 3037, 3038, 3039, 3040, 3041, 3042, 3043, 3044, 3045, 3046, 3047, 3048, 3049, 3050, 3051, 3052, 3053, 3054, 3055, 3056, 3057, 3058, 3059, 3060, 3061, 3062, 3063, 3064, 3065, 3066, 3067, 3068, 3069, 3070, 3071, 3072, 3073, 3074, 3075, 3076, 3077, 3078, 3079, 3080, 3081, 3082, 3083, 3084, 3085, 3086, 3087, 3088, 3089, 3090, 3091, 3092, 3093, 3094, 3095, 3096, 3097, 3098, 3099, 3100, 3101, 3102, 3103, 3113, 3114, 3115, 3116, 3117, 3118, 3119, 3120, 3121, 3305, 3306, 3307, 3308, 3309, 3310, 3311, 3312, 3313, 3314, 3315, 3316, 3317, 3318, 3319, 3320, 3321, 3322, 3323, 3324, 3325, 3326, 3327, 3328, 3329, 3330, 3331, 3332, 3333, 3334, 3335, 3336, 3337, 3338, 3339, 3340, 3341, 3342, 3343, 3344, 3345, 3346, 3347, 3348, 3349, 3350, 3351, 3352, 3353, 3354, 3355, 3356, 3357, 3358, 3359, 3360, 3361, 3362, 3363, 3364, 3365, 3366, 3367, 3368, 3369, 3370, 3371, 3372, 3373, 3374, 3375, 3376, 3377, 3378, 3379, 3380, 3381, 3382, 3383, 3384, 3385, 3386, 3387, 3388, 3389, 3390, 3391, 3392, 3393, 3394, 3395, 3396, 3397, 3398, 3399, 3400, 3401, 3402, 3403, 3404, 3405, 3406, 3407, 3408, 3409, 3410, 3411, 3412, 3413, 3414, 3415, 3416, 3417, 3418, 3419, 3420, 3421, 3422, 3423, 3424, 3425, 3426, 3427, 3428, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3437, 3438, 3439, 3440, 3441, 3442, 3443, 3444, 3445, 3446, 3447, 3448, 3449, 3450, 3451, 3452, 3453, 3454, 3455, 3479, 3480, 3481, 3482, 3483, 3484, 3485, 3486, 3487, 3488, 3489, 3490, 3491, 3492, 3493, 3522, 3523, 3524, 3525, 3526, 3527, 3528, 3529, 3530, 3531, 3532, 3533, 3534, 3535, 3536, 3537, 3538, 3539, 3540, 3549, 3550, 3551, 3552, 3553, 3554, 3555, 3556, 3628, 3629, 3630, 3631, 3632, 3633, 3634, 3635, 3636, 3637, 3638, 3639, 3640, 3641, 3642, 3643, 3644, 3645, 3646, 3647, 3648, 3649, 3650, 3651, 3652, 3653, 3654, 3655, 3656, 3657, 3658, 3659, 3660, 3661, 3662, 3663, 3664, 3665, 3666, 3667, 3668, 3669, 3670, 3671, 3672, 3673, 3674, 3675, 3676, 3677, 3678, 3679, 3680, 3681, 3682, 3683, 3684, 3685, 3686, 3687, 3688, 3689, 3690, 3691, 3692, 3693, 3694, 3695, 3696, 3697, 3698, 3755, 3756, 3757, 3758, 3759, 3760, 3761, 3762, 3763, 3764, 3765, 3766, 3767, 3768, 3769, 3770, 3771, 3772, 3773, 3774, 3775, 3776, 3777, 3778, 3779, 3780, 3781, 3782, 3783, 3784, 3785, 3786, 3787, 3788, 3789, 3790, 3791, 3792, 3793, 3794, 3795, 3796, 3797, 3798, 3799, 3800, 3801, 3802, 3803, 3804, 3805, 3806, 3807, 3808, 3809, and 3810, or a nucleotide sequence having at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99% sequence identity theretoThe methods may comprise the use of extended guide RNAs that comprise an RNA

extension at the 3′ end, wherein the RNA extension comprises the reverse transcription template sequence.

[0064] The methods may comprise the use of extended guide RNAs that comprise an RNA extension at the 5′ end, wherein the RNA extension comprises the reverse transcription template sequence.

[0065] The methods may comprise the use of extended guide RNAs that comprise an RNA extension at an intramolecular location in the guide RNA, wherein the RNA extension comprises the reverse transcription template sequence.

[0066] The methods may comprise the use of extended guide RNAs having one or more RNA extensions that are at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 200, at least 300, at least 400, or at least 500 nucleotides in length.

[0067] It should be appreciated that the foregoing concepts, and additional concepts discussed below, may be arranged in any suitable combination, as the present disclosure is not limited in this respect. Further, other advantages and novel features of the present disclosure will become apparent from the following detailed description of various non-limiting embodiments when considered in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0069] **FIG. 1A** provides a schematic of an exemplary process for introducing a single nucleotide change, and/or insertion, and/or deletion into a DNA molecule (e.g., a genome) using a fusion protein comprising a reverse transcriptase fused to a Cas9 protein in complex with an extended guide RNA molecule. In this embodiment, the guide RNA is extended at the 3′ end to include a reverse transcriptase template sequence. The schematic shows how a reverse transcriptase (RT) fused to a Cas9 nickase, in a complex with a guide RNA (gRNA), binds the DNA target site and nicks the PAM-containing DNA strand adjacent to the target nucleotide. The RT enzyme uses the nicked DNA as a primer for DNA synthesis from the gRNA, which is used as a template for the synthesis of a new DNA strand that encodes the

desired edit. The editing process shown may be referred to as target-primed reverse transcription editing (TRT editing) or equivalently, "prime editing."

[0070] FIG. 1B provides the same representation as in FIG. 1A, except that the prime editor complex is represented more generally as [napDNAbp]-[P]:PEgRNA or [P]-[napDNAbp]:PEgRNA, wherein "P" refers to any polymerase (e.g., a reverse transcriptase), "napDNAbp" refers to a nucleic acid programmable DNA binding protein (e.g., SpCas9), and "PEgRNA" refers to a prime editing guide RNA, and "]-[" refers to an optional linker. As described elsewhere, e.g., FIGs. 3A-3G, the PEgRNA comprises an 5′ extension arm comprising a primer binding site and a DNA synthesis template. Although not shown, it is contemplated that the extension arm of the PEgRNA (i.e., which comprises a primer binding site and a DNA synthesis template) can be DNA or RNA. The particular polymerase contemplated in this configuration will depend upon the nature of the DNA synthesis template. For instance, if the DNA synthesis template is RNA, then the polymerase case be an RNA-dependent DNA polymerase (e.g., reverse transcriptase). If the DNA synthesis template is DNA, then the polymerase can be a DNA-dependent DNA polymerase.

[0071] FIG. 1C provides a schematic of an exemplary process for introducing a single nucleotide change, and/or insertion, and/or deletion into a DNA molecule (e.g., a genome) using a fusion protein comprising a reverse transcriptase fused to a Cas9 protein in complex with an extended guide RNA molecule. In this embodiment, the guide RNA is extended at the 5′ end to include a reverse transcriptase template sequence. The schematic shows how a reverse transcriptase (RT) fused to a Cas9 nickase, in a complex with a guide RNA (gRNA), binds the DNA target site and nicks the PAM-containing DNA strand adjacent to the target nucleotide. The RT enzyme uses the nicked DNA as a primer for DNA synthesis from the gRNA, which is used as a template for the synthesis of a new DNA strand that encodes the desired edit. The editing process shown may be referred to as target-primed reverse transcription editing (TRT editing) or equivalently, "prime editing."

[0072] FIG. 1D provides the same representation as in FIG. 1C, except that the prime editor complex is represented more generally as [napDNAbp]-[P]:PEgRNA or [P]-[napDNAbp]:PEgRNA, wherein "P" refers to any polymerase (e.g., a reverse transcriptase), "napDNAbp" refers to a nucleic acid programmable DNA binding protein (e.g., SpCas9), and "PEgRNA" refers to a prime editing guide RNA, and "]-[" refers to an optional linker. As described elsewhere, e.g., FIGs. 3A-3G, the PEgRNA comprises an 3′ extension arm comprising a primer binding site and a DNA synthesis template. Although not shown, it is

contemplated that the extension arm of the PEgRNA (i.e., which comprises a primer binding site and a DNA synthesis template) can be DNA or RNA. The particular polymerase contemplated in this configuration will depend upon the nature of the DNA synthesis template. For instance, if the DNA synthesis template is RNA, then the polymerase case be an RNA-dependent DNA polymerase (e.g., reverse transcriptase). If the DNA synthesis template is DNA, then the polymerase can be a DNA-dependent DNA polymerase. In various embodiments, the PEgRNA can be engineered or synthesized to incorporate a DNA-based DNA synthesis template.

[0073] FIG. 1E is a schematic depicting an exemplary process of how the synthesized single strand of DNA (which comprises the desired nucleotide change) becomes resolved such that the desired nucleotide change is incorporated into the DNA. As shown, following synthesis of the edited strand (or "mutagenic strand"), equilibration with the endogenous strand, flap cleavage of the endogenous strand, and ligation leads to incorporation of the DNA edit after resolution of the mismatched DNA duplex through the action of endogenous DNA repair and/or replication processes.

[0074] FIG. 1F is a schematic showing that "opposite strand nicking" can be incorporated into the resolution method of **FIG. 1E** to help drive the formation of the desired product versus the reversion product. In opposite strand nicking, a second Cas9/gRNA complex is used to introduce a second nick on the opposite strand from the initial nicked strand. This induces the endogenous cellular DNA repair and/or replication processes to preferentially replace the unedited strand (i.e., the strand containing the second nick site).

[0075] FIG. 1G provides another schematic of an exemplary process for introducing a single nucleotide change, and/or insertion, and/or deletion into a DNA molecule (e.g., a genome) of a target locus using a nucleic acid programmable DNA binding protein (napDNAbp) complexed with an extended guide RNA. This process may be referred to as an embodiment of prime editing. The extended guide RNA comprises an extension at the 3′ or 5′ end of the guide RNA, or at an intramolecular location in the guide RNA. In step (a), the napDNAbp/gRNA complex contacts the DNA molecule and the gRNA guides the napDNAbp to bind to the target locus. In step (b), a nick in one of the strands of DNA (the R-loop strand, or the PAM-containing strand, or the non-target DNA strand, or the protospacer strand) of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand,

i.e., the strand that is not hybridized to the guide RNA sequence. In step (c), the 3′ end DNA strand interacts with the extended portion of the guide RNA in order to prime reverse transcription. In certain embodiments, the 3′ ended DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA. In step (d), a reverse transcriptase is introduced which synthesizes a single strand of DNA from the 3′ end of the primed site towards the 3′ end of the guide RNA. This forms a single-strand DNA flap comprising the desired nucleotide change (e.g., the single base change, insertion, or deletion, or a combination thereof). In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap such that the desired nucleotide change becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap that forms once the 3′ single strand DNA flap invades and hybridizes to the complementary sequence on the other strand. The process can also be driven towards product formation with second strand nicking, as exemplified in **FIG. 1F**. This process may introduce at least one or more of the following genetic changes: transversions, transitions, deletions, and insertions.

[0076] FIG. 1H is a schematic depicting the types of genetic changes that are possible with the prime editing processes described herein. The types of nucleotide changes achievable by prime editing include deletions (including short and long deletions), single-nucleotide changes (including transitions and transversions), inversions, and insertions (including short and long deletions).

[0077] FIG. 1I is a schematic depicting temporal second strand nicking exemplified by PE3b (PE3b = PE2 prime editor fusion protein + PEgRNA + second strand nicking guide RNA). Temporal second strand nicking is a variant of second strand nicking in order to facilitate the formation of the desired edited product. The "temporal" term refers to the fact that the second-strand nick to the unedited strand occurs only after the desired edit is installed in the edited strand. This avoids concurrent nicks on both strands to lead to double-stranded DNA breaks.

[0078] FIG. 1J depicts a variation of prime editing contemplated herein that replaces the napDNAbp (e.g., SpCas9 nickase) with any programmable nuclease domain, such as zinc finger nucleases (ZFN) or transcription activator-like effector nucleases (TALEN). As such, it is contemplated that suitable nucleases do not necessarily need to be "programmed" by a nucleic acid targeting molecule (such as a guide RNA), but rather, may be programmed by defining the specificity of a DNA-binding domain, such as and in particular, a nuclease. Just

as in prime editing with napDNAbp moieties, it is preferable that such alternative programmable nucleases be modified such that only one strand of a target DNA is cut. In other words, the programmable nucleases should function as nickases, preferably. Once a programmable nuclease is selected (e.g., a ZFN or a TALEN), then additional functionalities may be engineered into the system to allow it to operate in accordance with a prime editing-like mechanism. For example, the programmable nucleases may be modified by coupling (e.g., via a chemical linker) an RNA or DNA extension arm thereto, wherein the extension arm comprises a primer binding site (PBS) and a DNA synthesis template. The programmable nuclease may also be coupled (e.g., via a chemical or amino acid linker) to a polymerase, the nature of which will depend upon whether the extension arm is DNA or RNA. In the case of an RNA extension arm, the polymerase can be an RNA-dependent DNA polymerase (e.g., reverse transcriptase). In the case of a DNA extension arm, the polymerase can be a DNA-dependent DNA polymerase (e.g., a prokaryotic polymerase, including Pol I, Pol II, or Pol III, or a eukaryotic polymerase, including Pol a, Pol b, Pol g, Pol d, Pol e, or Pol z). The system may also include other functionalities added as fusions to the programmable nucleases, or added *in trans* to facilitate the reaction as a whole (e.g., (a) a helicase to unwind the DNA at the cut site to make the cut strand with the 3' end available as a primer, (b) a flap endonuclease (e.g., FEN1) to help remove the endogenous strand on the cut strand to drive the reaction towards replacement of the endogenous strand with the synthesized strand, or (c) a nCas9:gRNA complex to create a second site nick on the opposite strand, which may help drive the integration of the synthesize repair through favored cellular repair of the non-edited strand). In an analogous manner to prime editing with a napDNAbp, such a complex with an otherwise programmable nuclease could be used to synthesize and then install a newly synthesized replacement strand of DNA carrying an edit of interest permanently into a target site of DNA.

[0079] FIG. 1K depicts, in one embodiment, the anatomical features of a target DNA that may be edited by prime editing. The target DNA comprises a "non-target strand" and a "target strand." The target-strand is the strand that becomes annealed to the spacer of a PEgRNA of a prime editor complex that recognizes the PAM site (in this case, NGG, which is recognized by the canonical SpCas9-based prime editors) The target strand may also be referred to as the "non-PAM strand" or the "non-edit strand." By contrast, the non-target strand (i.e., the strand containing the protospacer and the PAM sequence of NGG) may be referred to as the "PAM-strand" or the "edit strand." In various embodiments, the nick site of

the PE complex will be in the protospacer on the PAM-strand (e.g., with the SpCas9-based PE). The location of the nick will be characteristic of the particular Cas9 that forms the PE. For example, with an SpCas9-based PE, the nick site in the phosphodiester bond between bases three ("-3" position relative to the position 1 of the PAM sequence) and four ("-4" position relative to position 1 of the PAM sequence). The nick site in the protospacer forms a free 3' hydroxyl group, which as seen in the following figures, complexes with the primer binding site of the extension arm of the PEgRNA and provides the substrate to begin polymerization of a single strand of DNA code for by the DNA synthesis template of the extension arm of the PEgRNA. This polymerization reaction is catalyzed by the polymerase (e.g., reverse transcriptase) of the PE fusion protein in the 5' to 3' direction. Polymerization terminates before reaching the gRNA core (e.g., by inclusion of a polymerization termination signal, or secondary structure, which functions to terminate the polymerization activity of PE), producing a single strand DNA flap that is extended from the original 3' hydroxyl group of the nicked PAM strand. The DNA synthesis template codes for a single strand DNA that is homologous to the endogenous 5'-ended single strand of DNA that immediately follows the nick site on the PAM strand and incorporates the desired nucleotide change (e.g., single base substitution, insertion, deletion, inversion). The position of the desired edit can be in any position following downstream of the nick site on the PAM strand, which can include position +1, +2, +3, +4 (the start of the PAM site), +5 (position 2 of the PAM site), +6 (position 3 of the PAM site), +7, +8, +9, +10, +11, +12, +13, +14, +15, +16, +17, +18, +19, +20, +21, +22, +23, +24, +25, +26, +27, +28, +29, +30, +31, +32, +33, +34, +35, +36, +37, +38, +39, +40, +41, +42, +43, +44, +45, +46, +47, +48, +49, +50, +51, +52, +53, +54, +55, +56, +57, +58, +59, +60, +61, +62, +63, +64, +65, +66, +67, +68, +69, +70, +71, +72, +73, +74, +75, +76, +77, +78, +79, +80, +81, +82, +83, +84, +85, +86, +87, +88, +89, +90, +91, +92, +93, +94, +95, +96, +97, +98, +99, +100, +101, +102, +103, +104, +105, +106, +107, +108, +109, +110, +111, +112, +113, +114, +115, +116, +117, +118, +119, +120, + 121, +122, +123, +124, +125, +126, +127, +128, +129, +130, +131, +132, +133, +134, +135, +136, +137, +138, +139, +140, +141, +142, +143, +144, +145, +146, +147, +148, +149, or +150, or more (relative to the downstream position of the nick site). Once the 3'end single stranded DNA (containing the edit of interest) replaces the endogenous 5' end single stranded DNA, the DNA repair and replication processes will result in permanent installation of the edit site on the PAM strand, and then correction of the mismatch on the non-PAM strand that will exist at the edit site. In this way, the edit will extend to both strands of DNA on the

target DNA site. It will be appreciated that reference to "edited strand" and "non-edited" strand only intends to delineate the strands of DNA involved in the PE mechanism. The "edited strand" is the strand that first becomes edited by replacement of the 5' ended single strand DNA immediately downstream of the nick site with the synthesized 3' ended single stranded DNA containing the desired edit. The "non-edited" strand is the strand pair with the edited strand, but which itself also becomes edited through repair and/or replication to be complementary to the edited strand, and in particular, the edit of interest.

[0080] FIG. IL depicts the mechanism of prime editing showing the anatomical features of the target DNA, prime editor complex, and the interaction between the PEgRNA and the target DNA. First, a prime editor comprising a fusion protein having a polymerase (e.g., reverse transcriptase) and a napDNAbp (e.g., SpCas9 nickase, e.g., a SpCas9 having a deactivating mutation in an HNH nuclease domain (e.g., H840A) or a deactivating mutation in a RuvC nuclease domain (D10A)) is complexed with a PEgRNA and DNA having a target DNA to be edited. The PEgRNA comprises a spacer, gRNA core (aka gRNA scaffold or gRNA backbone) (which binds to the napDNAbp), and an extension arm. The extension arm can be at the 3' end, the 5' end, or somewhere within the PEgRNA molecule. As shown, the extension arm is at the 3' end of the PEgRNA. The extension arm comprises in the 3' to 5' direction a primer binding site and a DNA synthesis template (comprising both an edit of interest and regions of homology (i.e., homology arms) that are homologous with the 5' ended single stranded DNA immediately following the nick site on the PAM strand. As shown, once the nick is introduced thereby producing a free 3' hydroxyl group immediately upstream of the nick site, the region immediately upstream of the nick site on the PAM strand anneals to a complementary sequence at the 3' end of the extension arm referred to as the "primer binding site," creating a short double-stranded region with an available 3' hydroxyl end, which forms a substrate for the polymerase of the prime editor complex. The polymerase (e.g., reverse transcriptase) then polymerase as strand of DNA from the 3' hydroxyl end to the end of the extension arm. The sequence of the single stranded DNA is coded for by the DNA synthesis template, which is the portion of the extension arm (i.e., excluding the primer binding site) that is "read" by the polymerase to synthesize new DNA. This polymerization effectively extends the sequence of the original 3' hydroxyl end of the initial nick site. The DNA synthesis template encodes a single strand of DNA that comprises not only the desired edit, but also regions that are homologous to the endogenous single strand of DNA immediately downstream of the nick site on the PAM strand. Next, the

encoded 3' ended single strand of DNA (i.e., the 3' single strand DNA flap) displaces the corresponding homologous endogenous 5'-ended single strand of DNA immediately downstream of the nick site on the PAM strand, forming a DNA intermediate having a 5'-ended single strand DNA flap, which is removed by the cell (e.g., by a flap endonuclease). The 3'-ended single strand DNA flap, which anneals to the complement of the endogenous 5'-ended single strand DNA flap, is ligated to the endogenous strand after the 5' DNA flap is removed. The desired edit in the 3' ended single strand DNA flap, now annealed and ligate, forms a mismatch with the complement strand, which undergoes DNA repair and/or a round of replication, thereby permanently installing the desired edit on both strands.

[0081] FIG. 2 shows three Cas complexes (SpCas9, SaCas9, and LbCas12a) that can be used in the herein described prime editors and their PAM, gRNA, and DNA cleavage features. The figure shows designs for complexes involving SpCas9, SaCas9, and LbCas12a.

[0082] FIGs. 3A-3F show designs for engineered 5′ prime editor gRNA (**FIG. 3A**), 3′ prime editor gRNA (**FIG. 3B**), and an intramolecular extension (**FIG. 3C**). The extended guide RNA (or extended gRNA) may also be referred to herein as PEgRNA or "prime editing guide RNA." **FIG. 3D** and **FIG. 3E** provide additional embodiments of 3′ and 5′ prime editor gRNAs (PEgRNAs), respectively. **FIG. 3F** illustrates the interaction between a 3′ end prime editor guide RNA with a target DNA sequence. The embodiments of **FIGs. 3A-3C** depict exemplary arrangements of the reverse transcription template sequence (i.e., or more broadly referred to as a DNA synthesis template, as indicated, since the RT is only one type of polymerase that may be used in the context of prime editors), the primer binding site, and an optional linker sequence in the extended portions of the 3′, 5′, and intramolecular versions, as well as the general arrangements of the spacer and core regions. The disclosed prime editing process is not limited to these configurations of extended guide RNAs. The embodiment of **FIG. 3D** provides the structure of an exemplary PEgRNA contemplated herein. The PEgRNA comprises three main component elements ordered in the 5′ to 3′ direction, namely: a spacer, a gRNA core, and an extension arm at the 3′ end. The extension arm may further be divided into the following structural elements in the 5′ to 3′ direction, namely: a primer binding site (A), an edit template (B), and a homology arm (C). In addition, the PEgRNA may comprise an optional 3′ end modifier region (e1) and an optional 5′ end modifier region (e2). Still further, the PEgRNA may comprise a transcriptional termination signal at the 3′ end of the PEgRNA (not depicted). These structural elements are further defined herein. The depiction of the structure of the PEgRNA is not meant to be limiting and embraces variations

in the arrangement of the elements. For example, the optional sequence modifiers (e1) and (e2) could be positioned within or between any of the other regions shown, and not limited to being located at the 3′ and 5′ ends. The PEgRNA could comprise, in certain embodiments, secondary RNA structure, such as, but not limited to, hairpins, stem/loops, toe loops, RNA-binding protein recruitment domains (e.g., the MS2 aptamer which recruits and binds to the MS2cp protein). For instance, such secondary structures could be position within the spacer, the gRNA core, or the extension arm, and in particular, within the e1 and/or e2 modifier regions. In addition to secondary RNA structures, the PEgRNAs could comprise (e.g., within the e1 and/or e2 modifier regions) a chemical linker or a poly(N) linker or tail, where "N" can be any nucleobase. In some embodiments (e.g., as shown in FIG. 72(c)), the chemical linker may function to prevent reverse transcription of the sgRNA scaffold or core. In addition, in certain embodiments (e.g., see FIG. 72(c)), the extension arm (3) could be comprised of RNA or DNA, and/or could include one or more nucleobase analogs (e.g., which might add functionality, such as temperature resilience). Still further, the orientation of the extension arm (3) can be in the natural 5′-to-3′ direction, or synthesized in the opposite orientation in the 3′-to-5′ direction (relative to the orientation of the PEgRNA molecule overall). It is also noted that one of ordinary skill in the art will be able to select an appropriate DNA polymerase, depending on the nature of the nucleic acid materials of the extension arm (i.e., DNA or RNA), for use in prime editing that may be implemented either as a fusion with the napDNAbp or as provided *in trans* as a separate moiety to synthesize the desired template-encoded 3′ single-strand DNA flap that includes the desired edit. For example, if the extension arm is RNA, then the DNA polymerase could be a reverse transcriptase or any other suitable RNA-dependent DNA polymerase. However, if the extension arm is DNA, then the DNA polymerase could be a DNA-dependent DNA polymerase. In various embodiments, provision of the DNA polymerase could be *in trans*, e.g., through the use of an RNA-protein recruitment domain (e.g., an MS2 hairpin installed on the PEgRNA (e.g., in the e1 or e2 region, or elsewhere and an MS2cp protein fused to the DNA polymerase, thereby co-localizing the DNA polymerase to the PEgRNA). It is also noted that the primer binding site does not generally form a part of the template that is used by the DNA polymerase (e.g., reverse transcriptase) to encode the resulting 3′ single-strand DNA flap that includes the desired edit. Thus, the designation of the "DNA synthesis template" refers to the region or portion of the extension arm (3) that is used as a template by the DNA polymerase to encode the desired 3′ single-strand DNA flap containing the edit and

regions of homology to the 5' endogenous single strand DNA flap that is replaced by the 3' single strand DNA strand product of prime editing DNA synthesis. In some embodiments, the DNA synthesis template includes the "edit template" and the "homology arm", or one or more homology arms, e.g., before and after the edit template. The edit template can be as small as a single nucleotide substitution, or it may be an insertion, or an inversion of DNA. In addition, the edit template may also include a deletion, which can be engineered by encoding homology arm that contains a desired deletion. In other embodiments, the DNA synthesis template may also include the e2 region or a portion thereof. For instance, if the e2 region comprises a secondary structure that causes termination of DNA polymerase activity, then it is possible that DNA polymerase function will be terminated before any portion of the e2 region is actual encoded into DNA. It is also possible that some or even all of the e2 region will be encoded into DNA. How much of e2 is actually used as a template will depend on its constitution and whether that constitution interrupts DNA polymerase function.

[0083] The embodiment of **FIG. 3E** provides the structure of another PEgRNA contemplated herein. The PEgRNA comprises three main component elements ordered in the 5′ to 3′ direction, namely: a spacer, a gRNA core, and an extension arm at the 3′ end. The extension arm may further be divided into the following structural elements in the 5′ to 3′ direction, namely: a primer binding site (A), an edit template (B), and a homology arm (C). In addition, the PEgRNA may comprise an optional 3′ end modifier region (e1) and an optional 5′ end modifier region (e2). Still further, the PEgRNA may comprise a transcriptional termination signal on the 3′ end of the PEgRNA (not depicted). These structural elements are further defined herein. The depiction of the structure of the PEgRNA is not meant to be limiting and embraces variations in the arrangement of the elements. For example, the optional sequence modifiers (e1) and (e2) could be positioned within or between any of the other regions shown, and not limited to being located at the 3′ and 5′ ends. The PEgRNA could comprise, in certain embodiments, secondary RNA structures, such as, but not limited to, hairpins, stem/loops, toe loops, RNA-binding protein recruitment domains (e.g., the MS2 aptamer which recruits and binds to the MS2cp protein). These secondary structures could be positioned anywhere in the PEgRNA molecule. For instance, such secondary structures could be position within the spacer, the gRNA core, or the extension arm, and in particular, within the e1 and/or e2 modifier regions. In addition to secondary RNA structures, the PEgRNAs could comprise (e.g., within the e1 and/or e2 modifier regions) a chemical linker or a poly(N) linker or tail, where "N" can be any nucleobase. In some embodiments (e.g., as

shown in FIG. 72(c)), the chemical linker may function to prevent reverse transcription of the sgRNA scaffold or core. In addition, in certain embodiments (e.g., see FIG. 72(c)), the extension arm (3) could be comprised of RNA or DNA, and/or could include one or more nucleobase analogs (e.g., which might add functionality, such as temperature resilience). Still further, the orientation of the extension arm (3) can be in the natural 5′-to-3′ direction, or synthesized in the opposite orientation in the 3′-to-5′ direction (relative to the orientation of the PEgRNA molecule overall). It is also noted that one of ordinary skill in the art will be able to select an appropriate DNA polymerase, depending on the nature of the nucleic acid materials of the extension arm (i.e., DNA or RNA), for use in prime editing that may be implemented either as a fusion with the napDNAbp or as provided in trans as a separate moiety to synthesize the desired template-encoded 3′ single-strand DNA flap that includes the desired edit. For example, if the extension arm is RNA, then the DNA polymerase could be a reverse transcriptase or any other suitable RNA-dependent DNA polymerase. However, if the extension arm is DNA, then the DNA polymerase could be a DNA-dependent DNA polymerase. In various embodiments, provision of the DNA polymerase could be in trans, e.g., through the use of an RNA-protein recruitment domain (e.g., an MS2 hairpin installed on the PEgRNA (e.g., in the e1 or e2 region, or elsewhere and an MS2cp protein fused to the DNA polymerase, thereby co-localizing the DNA polymerase to the PEgRNA). It is also noted that the primer binding site does not generally form a part of the template that is used by the DNA polymerase (e.g., reverse transcriptase) to encode the resulting 3′ single-strand DNA flap that includes the desired edit. Thus, the designation of the "DNA synthesis template" refers to the region or portion of the extension arm (3) that is used as a template by the DNA polymerase to encode the desired 3′ single-strand DNA flap containing the edit and regions of homology to the 5' endogenous single strand DNA flap that is replaced by the 3' single strand DNA strand product of prime editing DNA synthesis. In some embodiments, the DNA synthesis template includes the "edit template" and the "homology arm", or one or more homology arms, e.g., before and after the edit template. The edit template can be as small as a single nucleotide substitution, or it may be an insertion, or an inversion of DNA. In addition, the edit template may also include a deletion, which can be engineered by encoding homology arm that contains a desired deletion. In other embodiments, the DNA synthesis template may also include the e2 region or a portion thereof. For instance, if the e2 region comprises a secondary structure that causes termination of DNA polymerase activity, then it is possible that DNA polymerase function will be terminated before any portion of the

e2 region is actual encoded into DNA. It is also possible that some or even all of the e2 region will be encoded into DNA. How much of e2 is actually used as a template will depend on its constitution and whether that constitution interrupts DNA polymerase function.

[0084] The schematic of **FIG. 3F** depicts the interaction of a typical PEgRNA with a target site of a double stranded DNA and the concomitant production of a 3′ single stranded DNA flap containing the genetic change of interest. The double strand DNA is shown with the top strand (i.e., the target strand) in the 3′ to 5′ orientation and the lower strand (i.e., the PAM strand or non-target strand) in the 5′ to 3′ direction. The top strand comprises the complement of the "protospacer" and the complement of the PAM sequence and is referred to as the "target strand" because it is the strand that is target by and anneals to the spacer of the PEgRNA. The complementary lower strand is referred to as the "non-target strand" or the "PAM strand" or the "protospacer strand" since it contains the PAM sequence (e.g., NGG) and the protospacer. Although not shown, the PEgRNA depicted would be complexed with a Cas9 or equivalent domain of a prime editor fusion protein. As shown in the schematic, the spacer of the PEgRNA anneals to the complementary region of the protospacer on the target strand. This interaction forms as DNA/RNA hybrid between the spacer RNA and the complement of the protospacer DNA, and induces the formation of an R loop in the protospacer. As taught elsewhere herein, the Cas9 protein (not shown) then induces a nick in the non-target strand, as shown. This then leads to the formation of the 3′ ssDNA flap region immediately upstream of the nick site which, in accordance with *z*, interacts with the 3′ end of the PEgRNA at the primer binding site. The 3′ end of the ssDNA flap (i.e., the reverse transcriptase primer sequence) anneals to the primer binding site (A) on the PEgRNA, thereby priming reverse transcriptase. Next, reverse transcriptase (e.g., provided *in trans* or provided *cis* as a fusion protein, attached to the Cas9 construct) then polymerizes a single strand of DNA which is coded for by the DNA synthesis template (including the edit template (B) and homology arm (C)). The polymerization continues towards the 5′ end of the extension arm. The polymerized strand of ssDNA forms a ssDNA 3′ end flap which, as describe elsewhere (e.g., as shown in **FIG. 1G**), invades the endogenous DNA, displacing the corresponding endogenous strand (which is removed as a 5′ ended DNA flap of endogenous DNA), and installing the desired nucleotide edit (single nucleotide base pair change, deletions, insertions (including whole genes) through naturally occurring DNA repair/replication rounds.

[0085] FIG. 3G depicts yet another embodiment of prime editing contemplated herein. In particular, the top schematic depicts one embodiment of a prime editor (PE), which comprises a fusion protein of a napDNAbp (e.g., SpCas9) and a polymerase (e.g., a reverse transcriptase), which are joined by a linker. The PE forms a complex with a PEgRNA by binding to the gRNA core of the PEgRNA. In the embodiment shown, the PEgRNA is equipped with a 3′ extension arm that comprises, beginning at the 3′ end, a primer binding site (PBS) followed by a DNA synthesis template. The bottom schematic depicts a variant of a prime editor, referred to as a "*trans* prime editor (tPE)." In this embodiment, the DNA synthesis template and PBS are decoupled from the PEgRNA and presented on a separate molecule, referred to as a *trans* prime editor RNA template ("tPERT"), which comprises an RNA-protein recruitment domain (e.g., a MS2 hairpin). The PE itself is further modified to comprise a fusion to a rPERT recruiting protein ("RP"), which is a protein which specifically recognizes and binds to the RNA-protein recruitment domain. In the example where the RNA-protein recruitment domain is an MS2 hairpin, the corresponding rPERT recruiting protein can be MS2cp of the MS2 tagging system. The MS2 tagging system is based on the natural interaction of the MS2 bacteriophage coat protein ("MCP" or "MS2cp") with a stem-loop or hairpin structure present in the genome of the phage, i.e., the "MS2 hairpin" or "MS2 aptamer." In the case of *trans* prime editing, the RP-PE:gRNA complex "recruits" a tPERT having the appropriate RNA-protein recruitment domain to co-localize with the PE:gRNA complex, thereby providing the PBS and DNA synthesis template *in trans* for use in prime editing, as shown in the example depicted in FIG. 3H.

[0086] FIG. 3H depicts the process of *trans* prime editing. In this embodiment, the *trans* prime editor comprises a "PE2" prime editor (i.e., a fusion of a Cas9(H840A) and a variant MMLV RT) fused to an MS2cp protein (i.e., a type of recruiting protein that recognizes and binds to an MS2 aptamer) and which is complexed with an sgRNA (i.e., a standard guide RNA as opposed to a PEgRNA). The *trans* prime editor binds to the target DNA and nicks the nontarget strand. The MS2cp protein recruits a tPERT *in trans* through the specific interaction with the RNA-protein recruitment domain on the tPERT molecule. The tPERT becomes co-localized with the *trans* prime editor, thereby providing the PBS and DNA synthesis template functions *in trans* for use by the reverse transcriptase polymerase to synthesize a single strand DNA flap having a 3′ end and containing the desired genetic information encoded by the DNA synthesis template.

[0087] FIGs. 4A-4E demonstrate *in vitro* TPRT assays (i.e., prime editing assays). **FIG. 4A** is a schematic of fluorescently labeled DNA substrates gRNA templated extension by an RT enzyme, PAGE. **FIG. 4B** shows TPRT (i.e., prime editing) with pre-nicked substrates, dCas9, and 5′-extended gRNAs of differing synthesis template length. **FIG. 4C** shows the RT reaction with pre-nicked DNA substrates in the absence of Cas9. **FIG. 4D** shows TPRT (i.e., prime editing) on full dsDNA substrates with Cas9(H840A) and 5′-extended gRNAs. **FIG. 4E** shows a 3′-extended gRNA template with pre-nicked and full dsDNA substrates. All reactions are with M-MLV RT.

[0088] FIG. 5 shows *in vitro* validations using 5′-extended gRNAs with varying length synthesis templates. Fluorescently labeled (Cy5) DNA targets were used as substrates, and were pre-nicked in this set of experiments. The Cas9 used in these experiments is catalytically dead Cas9 (dCas9), and the RT used is Superscript III, a commercial RT derived from the Moloney-Murine Leukemia Virus (M-MLV). dCas9:gRNA complexes were formed from purified components. Then, the fluorescently labeled DNA substrate was added along with dNTPs and the RT enzyme. After 1 hour of incubation at 37 ºC, the reaction products were analyzed by denaturing urea-polyacrylamide gel electrophoresis (PAGE). The gel image shows extension of the original DNA strand to lengths that are consistent with the length of the reverse transcription template.

[0089] FIG. 6 shows *in vitro* validations using 5′-extended gRNAs with varying length synthesis templates, which closely parallels those shown in **FIG. 5**. However, the DNA substrates are not pre-nicked in this set of experiments. The Cas9 used in these experiments is a Cas9 nickase (SpyCas9 H840A mutant) and the RT used is Superscript III, a commercial RT derived from the Moloney-Murine Leukemia Virus (M-MLV). The reaction products were analyzed by denaturing urea-polyacrylamide gel electrophoresis (PAGE). As shown in the gel, the nickase efficiently cleaves the DNA strand when the standard gRNA is used (gRNA_0, lane 3).

[0090] FIG. 7 demonstrates that 3′ extensions support DNA synthesis and do not significantly effect Cas9 nickase activity. Pre-nicked substrates (black arrow) are near-quantitatively converted to RT products when either dCas9 or Cas9 nickase is used (lanes 4 and 5). Greater than 50% conversion to the RT product (red arrow) is observed with full substrates (lane 3). Cas9 nickase (SpyCas9 H840A mutant), catalytically dead Cas9 (dCas9) and Superscript III, a commercial RT derived from the Moloney-Murine Leukemia Virus (M-MLV) are used.

[0091] FIG. 8 demonstrates dual color experiments that were used to determine if the RT reaction preferentially occurs with the gRNA in cis (bound in the same complex). Two separate experiments were conducted for 5′-extended and 3′-extended gRNAs. Products were analyzed by PAGE. Product ratio calculated as (Cy3cis/Cy3trans) / (Cy5trans/Cy5cis).

[0092] FIGs. 9A-9D demonstrates a flap model substrate. **FIG. 9A** shows a dual-FP reporter for flap-directed mutagenesis. **FIG. 9B** shows stop codon repair in HEK cells. **FIG. 9C** shows sequenced yeast clones after flap repair. **FIG. 9D** shows testing of different flap features in human cells.

[0093] FIG. 10 demonstrates prime editing on plasmid substrates. A dual-fluorescent reporter plasmid was constructed for yeast (S. cerevisiae) expression. Expression of this construct in yeast produces only GFP. The *in vitro* prime editing reaction introduces a point mutation, and transforms the parent plasmid or an *in vitro* Cas9(H840A) nicked plasmid into yeast. The colonies are visualized by fluorescence imaging. Yeast dual-FP plasmid transformants are shown. Transforming the parent plasmid or an *in vitro* Cas9(H840A) nicked plasmid results in only green GFP expressing colonies. The prime editing reaction with 5′-extended or 3′-extended gRNAs produces a mix of green and yellow colonies. The latter express both GFP and mCherry. More yellow colonies are observed with the 3′-extended gRNA. A positive control that contains no stop codon is shown as well.

[0094] FIG. 11 shows prime editing on plasmid substrates similar to the experiment in **FIG. 10**, but instead of installing a point mutation in the stop codon, prime editing installs a single nucleotide insertion (left) or deletion (right) that repairs a frameshift mutation and allows for synthesis of downstream mCherry. Both experiments used 3′ extended gRNAs.

[0095] FIG. 12 shows editing products of prime editing on plasmid substrates, characterized by Sanger sequencing. Individually colonies from the TRT transformations were selected and analyzed by Sanger sequencing. Precise edits were observed by sequencing select colonies. Green colonies contained plasmids with the original DNA sequence, while yellow colonies contained the precise mutation designed by the prime editing gRNA. No other point mutations or indels were observed.

[0096] FIG. 13 shows the potential scope for the new prime editing technology is shown and compared to deaminase-mediated base editor technologies.

[0097] FIG. 14 shows a schematic of editing in human cells.

[0098] FIG. 15 demonstrates the extension of the primer binding site in gRNA.

[0099] FIG. 16 shows truncated gRNAs for adjacent targeting..

[0100] FIGs. 17A-17C are graphs displaying the % T to A conversion at the target nucleotide after transfection of components in human embryonic kidney (HEK) cells. **FIG. 17A** shows data, which presents results using an N-terminal fusion of wild type MLV reverse transcriptase to Cas9(H840A) nickase (32-amino acid linker). **FIG. 17B** is similar to **FIG. 17A**, but for C-terminal fusion of the RT enzyme. **FIG. 17C** is similar to **FIG. 17A** but the linker between the MLV RT and Cas9 is 60 amino acids long instead of 32 amino acids.

[0101] FIG. 18 shows high purity T to A editing at HEK3 site by high-throughput amplicon sequencing. The output of sequencing analysis displays the most abundant genotypes of edited cells.

[0102] FIG. 19 shows editing efficiency at the target nucleotide (blue bars) alongside indel rates (orange bars). WT refers to the wild type MLV RT enzyme. The mutant enzymes (M1 through M4) contain the mutations listed to the right. Editing rates were quantified by high throughput sequencing of genomic DNA amplicons.

[0103] FIG. 20 shows editing efficiency of the target nucleotide when a single strand nick is introduced in the complementary DNA strand in proximity to the target nucleotide. Nicking at various distances from the target nucleotide was tested (triangles). Editing efficiency at the target base pair (blue bars) is shown alongside the indel formation rate (orange bars). The "none" example does not contain a complementary strand nicking guide RNA. Editing rates were quantified by high throughput sequencing of genomic DNA amplicons.

[0104] FIG. 21 demonstrates processed high throughput sequencing data showing the desired T to A transversion mutation and general absence of other major genome editing byproducts.

[0105] FIG. 22 provides a schematic of an exemplary process for conducting targeted mutagenesis with an error-prone reverse transcriptase on a target locus using a nucleic acid programmable DNA binding protein (napDNAbp) complexed with an extended guide RNA, i.e., prime editing with an error-prone RT. This process may be referred to as an embodiment of prime editing for targeted mutagenesis. The extended guide RNA comprises an extension at the 3′ or 5′ end of the guide RNA, or at an intramolecular location in the guide RNA. In step (a), the napDNAbp/gRNA complex contacts the DNA molecule and the gRNA guides the napDNAbp to bind to the target locus to be mutagenized. In step (b), a nick in one of the strands of DNA of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand, i.e., the strand that is not hybridized to the guide RNA sequence. In step (c), the 3′ end DNA

strand interacts with the extended portion of the guide RNA in order to prime reverse transcription. In certain embodiments, the 3′ ended DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA. In step (d), an error-prone reverse transcriptase is introduced which synthesizes a mutagenized single strand of DNA from the 3′ end of the primed site towards the 3′ end of the guide RNA. Exemplary mutations are indicated with an asterisk "*". This forms a single-strand DNA flap comprising the desired mutagenized region. In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap (comprising the mutagenized region) such that the desired mutagenized region becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap that forms once the 3′ single strand DNA flap invades and hybridizes to the complementary sequence on the other strand. The process can also be driven towards product formation with second strand nicking, as exemplified in **FIG. 1F**. Following endogenous DNA repair and/or replication processes, the mutagenized region becomes incorporated into both strands of DNA of the DNA locus.

[0106] FIG. 23 is a schematic of gRNA design for contracting trinucleotide repeat sequences and trinucleotide repeat contraction with TPRT genome editing (i.e., prime editing). Trinucleotide repeat expansion is associated with a number of human diseases, including Huntington's disease, Fragile X syndrome, and Friedreich's ataxia. The most common trinucleotide repeat contains CAG triplets, though GAA triplets (Friedreich's ataxia) and CGG triplets (Fragile X syndrome) also occur. Inheriting a predisposition to expansion, or acquiring an already expanded parental allele, increases the likelihood of acquiring the disease. Pathogenic expansions of trinucleotide repeats could hypothetically be corrected using prime editing. A region upstream of the repeat region can be nicked by an RNA-guided nuclease, then used to prime synthesis of a new DNA strand that contains a healthy number of repeats (which depends on the particular gene and disease). After the repeat sequence, a short stretch of homology is added that matches the identity of the sequence adjacent to the other end of the repeat (red strand). Invasion of the newly synthesized strand, and subsequent replacement of the endogenous DNA with the newly synthesized flap, leads to a contracted repeat allele.

[0107] FIG. 24 is a schematic showing precise 10-nucleotide deletion with prime editing. A guide RNA targeting the HEK3 locus was designed with a reverse transcription template that

encodes a 10-nucleotide deletion after the nick site. Editing efficiency in transfected HEK cells was assessed using amplicon sequencing.

[0108] FIG. 25 is a schematic showing gRNA design for peptide tagging genes at endogenous genomic loci and peptide tagging with TPRT genome editing (i.e., prime editing). The FlAsH and ReAsH tagging systems comprise two parts: (1) a fluorophore-biarsenical probe, and (2) a genetically encoded peptide containing a tetracysteine motif, exemplified by the sequence FLNCCPGCCMEP (SEQ ID NO: 1). When expressed within cells, proteins containing the tetracysteine motif can be fluorescently labeled with fluorophore-arsenic probes (see ref: J. Am. Chem. Soc., 2002, 124 (21), pp 6063–6076. DOI: 10.1021/ja017687n). The "sortagging" system employs bacterial sortase enzymes that covalently conjugate labeled peptide probes to proteins containing suitable peptide substrates (see ref: Nat. Chem. Biol. 2007 Nov;3(11):707-8. DOI: 10.1038/nchembio.2007.31). The FLAG-tag (DYKDDDDK (SEQ ID NO: 2)), V5-tag (GKPIPNPLLGLDST (SEQ ID NO: 3)), GCN4-tag (EELLSKNYHLENEVARLKK (SEQ ID NO: 4)), HA-tag (YPYDVPDYA (SEQ ID NO: 5)), and Myc-tag (EQKLISEEDL (SEQ ID NO: 6)) are commonly employed as epitope tags for immunoassays. The pi-clamp encodes a peptide sequence (FCPF) that can by labeled with a pentafluoro-aromatic substrates (ref: Nat. Chem. 2016 Feb;8(2):120-8. doi: 10.1038/nchem.2413).

[0109] FIG. 26A shows precise installation of a His_6-tag and a FLAG-tag into genomic DNA. A guide RNA targeting the HEK3 locus was designed with a reverse transcription template that encodes either an 18-nt His-tag insertion or a 24-nt FLAG-tag insertion. Editing efficiency in transfected HEK cells was assessed using amplicon sequencing. Note that the full 24-nt sequence of the FLAG-tag is outside of the viewing frame (sequencing confirmed full and precise insertion). **FIG. 26B** shows a schematic outlining various applications involving protein/peptide tagging, including (a) rendering proteins soluble or insoluble, (b) changing or tracking the cellular localization of a protein, (c) extending the half-life of a protein, (d) facilitating protein purification, and (e) facilitating the detection of proteins.

[0110] FIG. 27 shows an overview of prime editing by installing a protective mutation in *PRNP* that prevents or halts the progression of prion disease. The PEgRNA sequences correspond to SEQ ID NO: 351 on the left (i.e., 5′ of the sgRNA scaffold) and SEQ ID NO 3864 on the right (i.e., 3′ of the sgRNA scaffold).

[0111] FIG. 28A is a schematic of PE-based insertion of sequences encoding RNA motifs. **FIG. 28B** is a list (not exhaustive) of some example motifs that could potentially be inserted, and their functions.

[0112] FIG. 29A is a depiction of a prime editor. **FIG. 29B** shows possible modifications to genomic, plasmid, or viral DNA directed by a PE. **FIG. 29C** shows an example scheme for insertion of a library of peptide loops into a specified protein (in this case GFP) via a library of PEgRNAs. **FIG. 29D** shows an example of possible programmable deletions of codons or N-, or C-terminal truncations of a protein using different PEgRNAs. Deletions would be predicted to occur with minimal generation of frameshift mutations.

[0113] FIG. 30 shows a possible scheme for iterative insertion of codons in a continual evolution system, such as PACE.

[0114] FIG. 31 is an illustration of an engineered gRNA showing the gRNA core, ~20nt spacer matching the sequence of the targeted gene, the reverse transcription template with immunogenic epitope nucleotide sequence and the primer binding site matching the sequence of the targeted gene.

[0115] FIG. 32 is a schematic showing using prime editing as a means to insert known immunogenicity epitopes into endogenous or foreign genomic DNA, resulting in modification of the corresponding proteins.

[0116] FIG. 33 is a schematic showing PEgRNA design for primer binding sequence insertions and primer binding insertion into genomic DNA using prime editing for determining off-target editing. In this embodiment, prime editing is conducted inside a living cell, a tissue, or an animal model. As a first step, an appropriate PEgRNA is designed. The top schematic shows an exemplary PEgRNA that may be used in this aspect. The spacer in the PEgRNA (labeled "protospacer") is complementary to one of the strands of the genomic target. The PE:PEgRNA complex (i.e., the PE complex) installs a single stranded 3′ end flap at the nick site which contains the encoded primer binding sequence and the region of homology (coded by the homology arm of the PEgRNA) that is complementary to the region just downstream of the cut site (in red). Through flap invasion and DNA repair/replication processes, the synthesized strand becomes incorporated into the DNA, thereby installing the primer binding site. This process can occur at the desired genomic target, but also at other genomic sites that might interact with the PEgRNA in an off-target manner (i.e., the PEgRNA guides the PE complex to other off-target sites due to the complementarity of the spacer region to other genomic sites that are not the intended genomic site). Thus, the primer

binding sequence may be installed not only at the desired genomic target, but at off-target genomic sites elsewhere in the genome. In order to detect the insertion of these primer binding sites at both the intended genomic target sites and the off-target genomic sites, the genomic DNA (post-PE) can be isolated, fragmented, and ligated to adapter nucleotides (shown in red). Next, PCR may be carried out with PCR oligonucleotides that anneal to the adapters and to the inserted primer binding sequence to amplify on-target and off-target genomic DNA regions into which the primer binding site was inserted by PE. High throughput sequencing then me be conducted to and sequence alignments to identify the insertion points of PE-inserted primer binding sequences at either the on-target site or at off-target sites.

[0117] FIG. 34 is a schematic showing the precise insertion of a gene with PE.

[0118] FIG. 35A is a schematic showing the natural insulin signaling pathway. **FIG. 35B** is a schematic showing FKBP12-tagged insulin receptor activation controlled by FK1012.

[0119] FIG. 36 shows small-molecule monomers. References: bumped FK506 mimic (**2**)[107]

[0120] FIG. 37 shows small-molecule dimers. References: FK1012 4[95,96]; FK1012 5[108]; FK1012 6[107]; AP1903 7[107]; cyclosporin A dimer 8[98]; FK506–cyclosporin A dimer (FkCsA) 9[100].

[0121] FIGs. 38A-38F provide an overview of prime editing and feasibility studies *in vitro* and in yeast cells. **FIG. 38A** shows the 75,122 known pathogenic human genetic variants in ClinVar (accessed July, 2019), classified by type. **FIG. 38B** shows that a prime editing complex consists of a prime editor (PE) protein containing an RNA-guided DNA-nicking domain, such as Cas9 nickase, fused to an engineered reverse transcriptase domain and complexed with a prime editing guide RNA (PEgRNA). The PE:PEgRNA complex binds the target DNA site and enables a large variety of precise DNA edits at a wide range of DNA positions before or after the target site's protospacer adjacent motif (PAM). **FIG. 38C** shows that upon DNA target binding, the PE:PEgRNA complex nicks the PAM-containing DNA strand. The resulting free 3′ end hybridizes to the primer- binding site of the PEgRNA. The reverse transcriptase domain catalyzes primer extension using the RT template of the PEgRNA, resulting in a newly synthesized DNA strand containing the desired edit (the 3′ flap).Equilibration between the edited 3′ flap and the unedited 5′ flap containing the original DNA, followed by cellular 5′ flap cleavage and ligation, and DNA repair or replication to resolve the heteroduplex DNA, results in stably edited DNA. **FIG. 38D** shows *in vitro* 5′-extended PEgRNA primer extension assays with pre-nicked dsDNA substrates containing 5′-

Cy5 labeled PAM strands, dCas9, and a commercial M-MLV RT variant (RT, Superscript III). dCas9 was complexed with PEgRNAs containing RT template of varying lengths, then added to DNA substrates along with the indicated components. Reactions were incubated at 37 °C for 1 hour, then analyzed by denaturing urea PAGE and visualized for Cy5 fluorescence. **FIG. 38E** shows primer extension assays performed as in **FIG. 38D** using 3′-extended PEgRNAs pre-complexed with dCas9 or Cas9 H840A nickase, and pre-nicked or non-nicked 5′-Cy5- labeled dsDNA substrates. **FIG. 38F** shows yeast colonies transformed with GFP–mCherry fusion reporter plasmids edited *in vitro* with PEgRNAs, Cas9 nickase, and RT. Plasmids containing nonsense or frameshift mutations between GFP and mCherry were edited with 5′-extended or 3′- extended PEgRNAs that restore mCherry translation via transversion mutation, 1-bp insertion, or 1-bp deletion. GFP and mCherry double-positive cells (yellow) reflect successful editing.

[0122] FIGs. 39A-39D show prime editing of genomic DNA in human cells by PE1 and PE2. **FIG. 39A** shows PEgRNAs contain a spacer sequence, a sgRNA scaffold, and a 3′ extension containing a primer- binding site (green) and a reverse transcription (RT) template (purple), which contains the edited base(s) (red). The primer-binding site hybridizes to the PAM-containing DNA strand immediately upstream of the site of nicking. The RT template is homologous to the DNA sequence downstream of the nick, with the exception of the encoded edit. **FIG. 39B** shows an installation of a T•A-to-A•T transversion edit at the HEK3 site in HEK293T cells using Cas9 H840A nickase fused to wild-type M-MLV reverse transcriptase (PE1) and PEgRNAs of varying primer- binding site lengths. **FIG. 39C**shows the use of an engineered pentamutant M-MLV reverse transcriptase (D200N, L603W, T306K, W313F, T330P) in PE2 substantially improves prime editing transversion efficiencies at five genomic sites in HEK293T cells, and small insertion and small deletion edits at HEK3. **FIG. 39D** is a comparison of PE2 editing efficiencies with varying RT template lengths at five genomic sites in HEK293T cells. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0123] FIGs. 40A-40C show PE3 and PE3b systems nick the non-edited strand to increase prime editing efficiency. **FIG. 40A** is an overview of the prime editing by PE3. After initial synthesis of the edited strand, DNA repair will remove either the newly synthesized strand containing the edit (3′ flap excision) or the original genomic DNA strand (5′ flap excision). 5′ flap excision leaves behind a DNA heteroduplex containing one edited strand and one non-edited strand. Mismatch repair machinery or DNA replication could resolve the

heteroduplex to give either edited or non-edited products. Nicking the non-edited strand favors repair of that strand, resulting in preferential generation of stable duplex DNA containing the desired edit. **FIG. 40B** shows the effect of complementary strand nicking on PE3-mediated prime editing efficiency and indel formation. "None" refers to PE2 controls, which do not nick the complementary strand. **FIG. 40C** is a comparison of editing efficiencies with PE2 (no complementary strand nick), PE3 (general complementary strand nick), and PE3b (edit-specific complementary strand nick). All editing yields reflect the percentage of total sequencing reads that contain the intended edit and do not contain indels among all treated cells, with no sorting. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0124] FIGS. 41A-41K show targeted insertions, deletions, and all 12 types of point mutations with PE3 at seven endogenous human genomic loci in HEK293T cells. **FIG. 41A** is a graph showing all 12 types of single- nucleotide transition and transversion edits from position +1 to +8 (counting the location of the PEgRNA-induced nick as between position +1 and -1) of the HEK3 site using a 10-nt RT template. **FIG. 41B** is a graph showing long-range PE3 transversion edits at the HEK3 site using a 34-nt RT template. **FIGs. 41C-41H** are graphs showing all 12 types of transition and transversion edits at various positions in the prime editing window for (**FIG. 41C**) RNF2, (**FIG. 41D**) FANCF, (**FIG. 41E**) EMX1, (**FIG. 41F**) RUNX1, (**FIG. 41G**) VEGFA, and (**FIG. 41H**) DNMT1. **FIG. 41I** is a graph showing targeted 1- and 3-bp insertions, and 1- and 3-bp deletions with PE3 at seven endogenous genomic loci. **FIG. 41J** is a graph showing the targeted precise deletions of 5 to 80 bp at the HEK3 target site. **FIG. 41K** is a graph showing a combination edits of insertions and deletions, insertions and point mutations, deletions and point mutations, and double point mutations at three endogenous genomic loci. All editing yields reflect the percentage of total sequencing reads that contain the intended edit and do not contain indels among all treated cells, with no sorting. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0125] FIGs. 42A-42H show the comparison of prime editing and base editing, and off-target editing by Cas9 and PE3 at known Cas9 off-target sites. **FIG. 42A** shows total C•G-to-T•A editing efficiency at the same target nucleotides for PE2, PE3, BE2max, and BE4max at endogenous HEK3, FANCF, and EMX1 sites in HEK293T cells. **FIG. 42B** shows indel frequency from treatments in **FIG. 42A. FIG. 42C** shows the editing efficiency of precise C•G-to-T•A edits (without bystander edits or indels) for PE2, PE3, BE2max, and BE4max at

HEK3, FANCF, and EMX1. For EMX1, precise PE combination edits of all possible combinations of C•G-to-T•A conversion at the three targeted nucleotides are also shown. **FIG. 42D** shows the total A•T-to-G•C editing efficiency for PE2, PE3, ABEdmax, and ABEmax at HEK3 and FANCF. **FIG. 42E** shows the precise A•T-to-G•C editing efficiency without bystander edits or indels for at HEK3 and FANCF. **FIG. 42F** shows indel frequency from treatments in **FIG. 42D**. **FIG. 42G** shows the average triplicate editing efficiencies (percentage sequencing reads with indels) in HEK293T cells for Cas9 nuclease at four on-target and 16 known off-target sites. The 16 off-target sites examined were the top four previously reported off-target sites[118,159] for each of the four on-target sites. For each on-target site, Cas9 was paired with a sgRNA or with each of four PEgRNAs that recognize the same protospacer. **FIG. 42H** shows the average triplicate on-target and off-target editing efficiencies and indel efficiencies (below in parentheses) in HEK293T cells for PE2 or PE3 paired with each PEgRNA in (**FIG. 42G**). On-target editing yields reflect the percentage of total sequencing reads that contain the intended edit and do not contain indels among all treated cells, with no sorting. Off-target editing yields reflect off-target locus modification consistent with prime editing. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0126] FIGs. 43A-43I show prime editing in various human cell lines and primary mouse cortical neurons, installation and correction of pathogenic transversion, insertion, or deletion mutations, and comparison of prime editing and HDR. **FIG. 43A** is a graph showing the installation (via T•A-to-A•T transversion) and correction (via A•T-to-T•A transversion) of the pathogenic E6V mutation in HBB in HEK293T cells. Correction either to wild-type HBB, or to HBB containing a silent mutation that disrupts the PEgRNA PAM, is shown. **FIG. 43B** is a graph showing the installation (via 4-bp insertion) and correction (via 4-bp deletion) of the pathogenic HEXA 1278+TATC allele in HEK293T cells. Correction either to wild-type HEXA, or to HEXA containing a silent mutation that disrupts the PEgRNA PAM, is shown. **FIG. 43C** is a graph showing the installation of the protective G127V variant in PRNP in HEK293T cells via G•C-to-T•A transversion. **FIG. 43D** is a graph showing prime editing in other human cell lines including K562 (leukemic bone marrow cells), U2OS (osteosarcoma cells), and HeLa (cervical cancer cells). **FIG. 43E** is a graph showing the installation of a G•C-to-T•A transversion mutation in DNMT1 of mouse primary cortical neurons using a dual split-intein PE3 lentivirus system, in which the N-terminal half is Cas9 (1-573) fused to N-intein and through a P2A self-cleaving peptide to GFP–KASH, and the C-terminal half is

the C-intein fused to the remainder of PE2. PE2 halves are expressed from a human synapsin promoter that is highly specific for mature neurons. Sorted values reflect editing or indels from GFP-positive nuclei, while unsorted values are from all nuclei. **FIG. 43F** is a comparison of PE3 and Cas9-mediated HDR editing efficiencies at endogenous genomic loci in HEK293T cells. **FIG. 43G** is a comparison of PE3 and Cas9-mediated HDR editing efficiencies at endogenous genomic loci in K562, U2OS, and HeLa cells. **FIG. 43H** is a comparison of PE3 and Cas9-mediated HDR indel byproduct generation in HEK293T, K562, U2OS, and HeLa cells. **FIG. 43I** shows targeted insertion of a His6 tag (18 bp), FLAG epitope tag (24 bp), or extended LoxP site (44 bp) in HEK293T cells by PE3. All editing yields reflect the percentage of total sequencing reads that contain the intended edit and do not contain indels among all treated cells. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0127] FIGs. 44A-44G show *in vitro* prime editing validation studies with fluorescently labeled DNA substrates. **FIG. 44A** shows electrophoretic mobility shift assays with dCas9, 5′-extended PEgRNAs and 5′-Cy5-labeled DNA substrates. PEgRNAs 1 through 5 contain a 15-nt linker sequence (linker A for PEgRNA 1, linker B for PEgRNAs 2 through 5) between the spacer and the PBS, a 5-nt PBS sequence, and RT templates of 7 nt (PEgRNAs 1 and 2), 8 nt (PEgRNA 3), 15 nt (PEgRNA 4), and 22 nt (PEgRNA 5). PEgRNAs are those used in **FIG. 44E** and **44F**; full sequences are listed in Tables 2A-2C. **FIG. 44B** shows *in vitro* nicking assays of Cas9 H840A using 5′-extended and 3′-extended PEgRNAs. **FIG. 44C** shows Cas9-mediated indel formation in HEK293T cells at HEK3 using 5′-extended and 3′-extended PEgRNAs. **FIG. 44D** shows an overview of prime editing *in vitro* biochemical assays. 5′-Cy5-labeled pre-nicked and non-nicked dsDNA substrates were tested. sgRNAs, 5′-extended PEgRNAs, or 3′-extended PEgRNAs were pre-complexed with dCas9 or Cas9 H840A nickase, then combined with dsDNA substrate, M-MLV RT, and dNTPs. Reactions were allowed to proceed at 37 °C for 1 hour prior to separation by denaturing urea PAGE and visualization by Cy5 fluorescence. **FIG. 44E** shows primer extension reactions using 5′-extended PEgRNAs, pre-nicked DNA substrates, and dCas9 lead to significant conversion to RT products. **FIG. 44F** shows primer extension reactions using 5′-extended PEgRNAs as in **FIG. 44B**, with non-nicked DNA substrate and Cas9 H840A nickase. Product yields are greatly reduced by comparison to pre-nicked substrate. **FIG. 44G** shows an *in vitro* primer extension reaction using a 3′-PEgRNA generates a single apparent product by denaturing urea PAGE. The RT product band was excised, eluted from the gel, then subjected to

homopolymer tailing with terminal transferase (TdT) using either dGTP or dATP. Tailed products were extended by poly-T or poly-C primers, and the resulting DNA was sequenced. Sanger traces indicate that three nucleotides derived from the gRNA scaffold were reverse transcribed (added as the final 3′ nucleotides to the DNA product). Note that in mammalian cell prime editing experiments, PEgRNA scaffold insertion is much rarer than in vitro (**FIGs. 56A-56D**), potentially due to the inability of the tethered reverse transcriptase to access the Cas9-bound guide RNA scaffold, and/or cellular excision of mismatched 3′ ends of 3′ flaps containing PEgRNA scaffold sequences.

[0128] FIGs. 45A-45G show cellular repair in yeast of 3′ DNA flaps from *in vitro* prime editing reactions. **FIG. 45A** shows that dual fluorescent protein reporter plasmids contain GFP and mCherry open reading frames separated by a target site encoding an in-frame stop codon, a +1 frameshift, or a -1 frameshift. Prime editing reactions were carried out *in vitro* with Cas9 H840A nickase, PEgRNA, dNTPs, and M-MLV reverse transcriptase, and then transformed into yeast. Colonies that contain unedited plasmids produce GFP but not mCherry. Yeast colonies containing edited plasmids produce both GFP and mCherry as a fusion protein. **FIG. 45B** shows an overlay of GFP and mCherry fluorescence for yeast colonies transformed with reporter plasmids containing a stop codon between GFP and mCherry (unedited negative control, top), or containing no stop codon or frameshift between GFP and mCherry (pre-edited positive control, bottom). **FIGs. 45C-45F** show a visualization of mCherry and GFP fluorescence from yeast colonies transformed with *in vitro* prime editing reaction products. **FIG. 45C** shows a stop codon correction via T•A-to- A•T transversion using a 3′-extended PEgRNA, or a 5′-extended PEgRNA, as shown in **FIG. 45D**. **FIG. 45E** shows a +1 frameshift correction via a 1-bp deletion using a 3′-extended PEgRNA. **FIG. 45F** shows a -1 frameshift correction via a 1-bp insertion using a 3′-extended PEgRNA. **FIG. 45G** shows Sanger DNA sequencing traces from plasmids isolated from GFP-only colonies in **FIG. 45B** and GFP and mCherry double-positive colonies in **FIG. 45C**.

[0129] FIGs. 46A-46F show correct editing versus indel generation with PE1. **FIG. 46A** shows T•A-to-A•T transversion editing efficiency and indel generation by PE1 at the +1 position of HEK3 using PEgRNAs containing 10-nt RT templates and a PBS sequences ranging from 8-17 nt. **FIG. 46B** shows G•C-to-T•A transversion editing efficiency and indel generation by PE1 at the +5 position of EMX1 using PEgRNAs containing 13-nt RT templates and a PBS sequences ranging from 9- 17 nt. **FIG. 46C** shows G•C-to-T•A transversion editing efficiency and indel generation by PE1 at the +5 position of FANCF

using PEgRNAs containing 17-nt RT templates and a PBS sequences ranging from 8-17 nt. **FIG. 46D** shows C•G-to-A•T transversion editing efficiency and indel generation by PE1 at the +1 position of RNF2 using PEgRNAs containing 11-nt RT templates and a PBS sequences ranging from 9-17 nt. **FIG. 46E** shows G•C-to-T•A transversion editing efficiency and indel generation by PE1 at the +2 position of HEK4 using PEgRNAs containing 13-nt RT templates and a PBS sequences ranging from 7-15 nt. **FIG. 46F** shows PE1-mediated +1 T deletion, +1 A insertion, and +1 CTT insertion at the HEK3 site using a 13-nt PBS and 10-nt RT template. Sequences of PEgRNAs are those used in **FIG. 39C** (see Tables 3A-3R). Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0130] FIGs. 47A-47S show the evaluation of M-MLV RT variants for prime editing. **FIG. 47A** shows the abbreviations for prime editor variants used in this figure. **FIG. 47B** shows targeted insertion and deletion edits with PE1 at the HEK3 locus. **FIGs. 47C-47H** show a comparison of 18 prime editor constructs containing M-MLV RT variants for their ability to install a +2 G•C-to-C•G transversion edit at HEK3 as shown in **FIG. 47C**, a 24-bp FLAG insertion at HEK3 as shown in **FIG. 47D**, a +1 C•G-to-A•T transversion edit at RNF2 as shown in **FIG. 47E**, a +1 G•C-to-C•G transversion edit at EMX1 as shown in **FIG. 47F**, a +2 T•A-to-A•T transversion edit at *HBB* as shown in **FIG. 47G**, and a +1 G•C-to-C•G transversion edit at FANCF as shown in **FIG. 47H**. **FIGs. 47I-47N** show a comparison of four prime editor constructs containing M-MLV variants for their ability to install the edits shown in **FIGs. 47C-47H** in a second round of independent experiments. **FIGs. 47O-47S** show PE2 editing efficiency at five genomic loci with varying PBS lengths. **FIG. 47O** shows a +1 T•A-to-A•T variation at HEK3. **FIG. 47P** shows a +5 G•C-to-T•A variation at EMX1. **FIG. 47Q** shows a +5 G•C-to-T•A variation at FANCF. **FIG. 47R** shows a +1 C•G-to-A•T variation at RNF2. **FIG. 47S** shows a +2 G•C-to-T•A variation at HEK4. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0131] FIGs. 48A-48C show design features of PEgRNA PBS and RT template sequences. **FIG. 48A** shows PE2-mediated +5 G•C-to-T•A transversion editing efficiency (blue line) at VEGFA in HEK293T cells as a function of RT template length. Indels (gray line) are plotted for comparison. The sequence below the graph shows the last nucleotide templated for synthesis by the PEgRNA. G nucleotides (templated by a C in the PEgRNA) are highlighted; RT templates that end in C should be avoided during PEgRNA design to maximize prime editing efficiencies. **FIG. 48B** shows +5 G•C-to-T•A transversion editing and indels for DNMT1 as in **FIG. 48A**. **FIG. 48C** shows +5 G•C-to-T•A transversion editing and indels for

RUNX1 as in **FIG. 48A**. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0132] FIGs. 49A-49B show the effects of PE2, PE2 R110S K103L, Cas9 H840A nickase, and dCas9 on cell viability. HEK293T cells were transfected with plasmids encoding PE2, PE2 R110S K103L, Cas9 H840A nickase, or dCas9, together with a HEK3-targeting PEgRNA plasmid. Cell viability was measured every 24 hours post-transfection for 3 days using the CellTiter-Glo 2.0 assay (Promega). **FIG. 49A** shows viability, as measured by luminescence, at 1, 2, or 3 days post-transfection. Values and error bars reflect the mean and s.e.m. of three independent biological replicates each performed in technical triplicate. **FIG. 49B** shows percent editing and indels for PE2, PE2 R110S K103L, Cas9 H840A nickase, or dCas9, together with a HEK3-targeting PEgRNA plasmid that encodes a +5 G to A edit. Editing efficiencies were measured on day 3 post-transfection from cells treated alongside of those used for assaying viability in **FIG. 49A**. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0133] FIGs. 50A-50B show PE3-mediated *HBB* E6V correction and *HEXA* 1278+TATC correction by various PEgRNAs. **FIG. 50A** shows a screen of 14 PEgRNAs for correction of the *HBB* E6V allele in HEK293T cells with PE3. All PEgRNAs evaluated convert the *HBB* E6V allele back to wild-type *HBB* without the introduction of any silent PAM mutation. **FIG. 50B** shows a screen of 41 PEgRNAs for correction of the *HEXA* 1278+TATC allele in HEK293T cells with PE3 or PE3b. Those PEgRNAs labeled *HEXA*s correct the pathogenic allele by a shifted 4-bp deletion that disrupts the PAM and leaves a silent mutation. Those PEgRNAs labeled *HEXA* correct the pathogenic allele back to wild-type. Entries ending in "b" use an edit-specific nicking sgRNA in combination with the PEgRNA (the PE3b system). Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0134] FIGs. 51A-51F show a PE3 activity in human cell lines and a comparison of PE3 and Cas9-initiated HDR. Efficiency of generating the correct edit (without indels) and indel frequency for PE3 and Cas9-initiated HDR in HEK293T cells as shown in **FIG. 51A**, K562 cells as shown in **FIG. 51B**, U2OS cells as shown in **FIG. 51C**, and HeLa cells as shown in **FIG. 51D**. Each bracketed editing comparison installs identical edits with PE3 and Cas9-initiated HDR. Non- targeting controls are PE3 and a PEgRNA that targets a non-target locus. **FIG. 51E** shows control experiments with non-targeting PEgRNA+PE3, and with dCas9+sgRNA, compared with wild- type Cas9 HDR experiments confirming that ssDNA donor HDR template, a common contaminant that artificially elevates apparent HDR

efficiencies, does not contribute to the HDR measurements in **FIGs. 51A-51D**. **FIG. 51F** shows example HEK3 site allele tables from genomic DNA samples isolated from K562 cells after editing with PE3 or with Cas9-initiated HDR. Alleles were sequenced on an Illumina MiSeq and analyzed with CRISPResso2[178]. The reference HEK3 sequence from this region is at the top. Allele tables are shown for a non-targeting PEgRNA negative control, a +1 CTT insertion at HEK3 using PE3, and a +1 CTT insertion at HEK3 using Cas9-initiated HDR. Allele frequencies and corresponding Illumina sequencing read counts are shown for each allele. All alleles observed with frequency ≥0.20% are shown. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0135] FIGs. 52A-52D show distribution by length of pathogenic insertions, duplications, deletions, and indels in the ClinVar database. The ClinVar variant summary was downloaded from NCBI July 15, 2019. The lengths of reported insertions, deletions, and duplications were calculated using reference and alternate alleles, variant start and stop positions, or appropriate identifying information in the variant name. Variants that did not report any of the above information were excluded from the analysis. The lengths of reported indels (single variants that include both insertions and deletions relative to the reference genome) were calculated by determining the number of mismatches or gaps in the best pairwise alignment between the reference and alternate alleles.

[0136] FIGs. 53A-53B show FACS gating examples for GFP-positive cell sorting. Below are examples of original batch analysis files outlining the sorting strategy used for generating *HEXA* 1278+TATC and *HBB* E6V HEK293T cell lines. The image data was generated on a Sony LE-MA900 cytometer using Cell Sorter Software v. 3.0.5. Graphic 1 shows gating plots for cells that do not express GFP. Graphic 2 shows an example sort of P2A–GFP-expressing cells used for isolating the *HBB* E6V HEK293T cell lines. HEK293T cells were initially gated on population using FSC-A/BSC-A (Gate A), then sorted for singlets using FSC-A/FSC-H (Gate B). Live cells were sorted for by gating DAPI-negative cells (Gate C). Cells with GFP fluorescence levels that were above those of the negative-control cells were sorted for using EGFP as the fluorochrome (Gate D). **FIG. 53A** shows HEK293T cells (GFP-negative). **FIG. 53B** shows a representative plot of FACS gating for cells expressing PE2–P2A–GFP. **FIG. 53C** shows the genotypes for HEXA 1278+TATC homozygote HEK293T cells. **FIG. 53D** shows allele tables for HBB E6V homozygote HEK293T cell lines.

[0137] FIG. 54 is a schematic which summarizes the PEgRNA cloning procedure.

[0138] **FIGs. 55A-55G** are schematics of PEgRNA designs. **FIG. 55A** shows a simple diagram of PEgRNA with domains labeled (left) and bound to nCas9 at a genomic site (right). **FIG. 55B** shows various types of modifications to PEgRNA which are anticipated to increase activity. **FIG. 55C** shows modifications to PEgRNA to increase transcription of longer RNAs via promoter choice and 5′, 3′ processing and termination. **FIG. 55D** shows the lengthening of the P1 system, which is an example of a scaffold modification. **FIG. 55E** shows that the incorporation of synthetic modifications within the template region, or elsewhere within the PEgRNA, could increase activity. **FIG. 55F** shows that a designed incorporation of minimal secondary structure within the template could prevent formation of longer, more inhibitory, secondary structure. **FIG. 55G** shows a split PEgRNA with a second template sequence anchored by an RNA element at the 3′ end of the PEgRNA (left). Incorporation of elements at the 5′ or 3′ ends of the PEgRNA could enhance RT binding.

[0139] **FIGs. 56A-56D** show the incorporation of PEgRNA scaffold sequence into target loci. HTS data were analyzed for PEgRNA scaffold sequence insertion as described in FIGs. 60A-60B. **FIG. 56A** shows an analysis for the EMX1 locus. Shown is the % of total sequencing reads containing one or more PEgRNA scaffold sequence nucleotides within an insertion adjacent to the RT template (left); the percentage of total sequencing reads containing a PEgRNA scaffold sequence insertion of the specified length (middle); and the cumulative total percentage of PEgRNA insertion up to and including the length specified on the X axis. **FIG. 56B** shows the same as **FIG. 56A,** but for FANCF. **FIG. 56C** shows the same as in **FIG. 56A** but for HEK3. **FIG. 56D** shows the same as **FIG. 56A** but for RNF2. Values and error bars reflect the mean and s.d. of three independent biological replicates.

[0140] **FIGs. 57A-57I** show the effects of PE2, PE2-dRT, and Cas9 H840A nickase on transcriptome-wide RNA abundance. Analysis of cellular RNA, depleted for ribosomal RNA, isolated from HEK293T cells expressing PE2, PE2-dRT, or Cas9 H840A nickase and a PRNP-targeting or HEXA-targeting PEgRNA. RNAs corresponding to 14,410 genes and 14,368 genes were detected in PRNP and HEXA samples, respectively. **FIGs. 57A-57F** show Volcano plot displaying the -log10 FDR-adjusted p-value vs. log2-fold change in transcript abundance for Aeach RNA, comparing (**FIG. 57A**) PE2 vs. PE2-dRT with PRNP-targeting PEgRNA, (**FIG. 57B**) PE2 vs. Cas9 H840A with PRNP-targeting PEgRNA, (**FIG. 57C**) PE2-dRT vs. Cas9 H840A with PRNP-targeting PEgRNA, (**FIG. 57D**) PE2 vs. PE2-dRT with HEXA-targeting PEgRNA, (**FIG. 57E**) PE2 vs. Cas9 H840A with HEXA-targeting PEgRNA, (**FIG. 57F**) PE2-dRT vs. Cas9 H840A with HEXA-targeting PEgRNA. Red dots

indicate genes that show ≥2-fold change in relative abundance that are statistically significant (FDR-adjusted $p < 0.05$). **FIGs. 57G-57I** are Venn diagrams of upregulated and downregulated transcripts (≥2-fold change) comparing PRNP and HEXA samples for (**FIG. 57G**) PE2 vs PE2-dRT, (**FIG. 57H**) PE2 vs. Cas9 H840A, and (**FIG. 57I**) PE2-dRT vs. Cas9 H840A.

[0141] FIG. 58 shows representative FACS gating for neuronal nuclei sorting. Nuclei were sequentially gated on the basis of DyeCycle Ruby signal, FSC/SSC ratio, SSC-Width/SSC-height ratio, and GFP/DyeCycle ratio.

[0142] FIGs. 59A-59F show the protocol for cloning 3′-extended PEgRNAs into mammalian U6 expression vectors by Golden Gate assembly. **FIG. 59A** shows the cloning overview. **FIG. 59B** shows 'Step 1: Digest pU6-PEgRNA-GG-Vector plasmid (component 1)'. **FIG. 59C** shows 'Steps 2 and 3: Order and anneal oligonucleotide parts (components 2, 3, and 4)'. **FIG. 59D** shows 'Step 2.b.ii.: sgRNA scaffold phosphorylation (unnecessary if oligonucleotides were purchased phosphorylated)'. **FIG. 59E** shows 'Step 4: PEgRNA assembly'. **FIG. 59F** shows 'Steps 5 and 6: Transformation of assembled plasmids'. **FIG. 59G** shows a diagram summarizing the PEgRNA cloning protocol.

[0143] FIGs. 60A-60B show the Python script for quantifying PEgRNA scaffold integration. A custom python script was generated to characterize and quantify PEgRNA insertions at target genomic loci. The script iteratively matches text strings of increasing length taken from a reference sequence (guide RNA scaffold sequence) to the sequencing reads within fastq files, and counts the number of sequencing reads that match the search query. Each successive text string corresponds to an additional nucleotide of the guide RNA scaffold sequence. Exact length integrations and cumulative integrations up to a specified length were calculated in this manner. At the start of the reference sequence, 5 to 6 bases of the 3′ end of the new DNA strand synthesized by the reverse transcriptase are included to ensure alignment and accurate counting of short slices of the sgRNA.

[0144] FIG. 61 is a graph showing the percent of total sequencing reads with the specified edit for SaCas9(N580A)-MMLV RT HEK3 +6 C>A. The values for the correct edits as well as indels are shown.

[0145] FIGs. 62A-62B show the importance of the protospacer for efficient installation of a desired edit at a precise location with prime editing. **FIG. 62A** is a graph showing the percent of total sequencing reads with target T•A base pairs converted to A•T for various HEK3 loci. **FIG. 62B** is a sequence analysis showing the same.

[0146] FIG. 63 is a graph showing SpCas9 PAM variants in PAM editing (N=3). The percent of total sequencing reads with the targeted PAM edit is shown for SpCas9(H840A)-VRQR-MMLV RT, where NGA > NTA, and for SpCas9(H840A)-VRER-MMLV RT, where NGCG > NTCG. The PEgRNA primer binding site (PBS) length, RT template (RT) length, and PE system used are listed.

[0147] FIG. 64 is a schematic showing the introduction of various site-specific recombinase (SSR) targets into the genome using PE. (a) provides a general schematic of the insertion of a recombinase target sequence by a prime editor. (b) shows how a single SSR target inserted by PE can be used as a site for genomic integration of a DNA donor template. (c) shows how a tandem insertion of SSR target sites can be used to delete a portion of the genome. (d) shows how a tandem insertion of SSR target sites can be used to invert a portion of the genome. (e) shows how the insertion of two SSR target sites at two distal chromosomal regions can result in chromosomal translocation. (f) shows how the insertion of two different SSR target sites in the genome can be used to exchange a cassette from a DNA donor template. See Example 17 for further details.

[0148] FIG. 65 shows in 1) the PE-mediated synthesis of a SSR target site in a human cell genome and 2) the use of that SSR target site to integrate a DNA donor template comprising a GFP expression marker. Once successfully integrated, the GFP causes the cell to fluoresce. See Example 17 for further details.

[0149] FIG. 66 depicts one embodiment of a prime editor being provided as two PE half proteins which regenerate as whole prime editor through the self-splicing action of the split-intein halves located at the end or beginning of each of the prime editor half proteins.

[0150] FIG. 67 depicts the mechanism of intein removal from a polypeptide sequence and the reformation of a peptide bond between the N-terminal and the C-terminal extein sequences. (a) depicts the general mechanism of two half proteins each containing half of an intein sequence, which when in contact within a cell result in a fully-functional intein which then undergoes self-spicing and excision. The process of excision results in the formation of a peptide bond between the N-terminal protein half (or the "N extein") and the C-terminal protein half (or the "C extein") to form a whole, single polypeptide comprising the N extein and the C extein portions. In various embodiments, the N extein may correspond to the N-terminal half of a split prime editor fusion protein and the C extein may correspond to the C-terminal half of a split prime editor. (b) shows a chemical mechanism of intein excision and the reformation of a peptide bond that joins the N extein half (the red-colored half) and the C

extein half (the blue-colored half). Excision of the split inteins (i.e., the N intein and the C intein in the split intein configuration) may also be referred to as "trans splicing" as it involves the splicing action of two separate components provided *in trans*.

[0151] FIG. 68A demonstrates that delivery of both split intein halves of SpPE (SEQ ID NO: 762) at the linker maintains activity at three test loci when co-transfected into HEK293T cells.

[0152] FIG. 68B demonstrates that delivery of both split intein halves of SaPE2 (e.g., SEQ ID NO: 443 and SEQ ID NO: 450) recapitulate activity of full length SaPE2 (SEQ ID NO: 134) when co-transfected into HEK293T cells. Residues indicated in quotes are the sequence of amino acids 741-743 in SaCas

[0153] 9 (first residues of the C-terminal extein) which are important for the intein trans splicing reaction. 'SMP' are the native residues, which we also mutated to the 'CFN' consensus splicing sequence. The consensus sequence is shown to yield the highest reconstitution as measured by prime editing percentage.

[0154] FIG. 68C provides data showing that various disclosed PE ribonucleoprotein complexes (PE2 at high concentration, PE3 at high concentration and PE3 at low concentration) can be delivered in this manner.

[0155] FIG. 69 shows a bacteriophage plaque assay to determine PE effectiveness in PANCE. Plaques (dark circles) indicate phage able to successfully infect *E. coli*. Increasing concentration of L-rhamnose results in increased expression of PE and an increase in plaque formation. Sequencing of plaques revealed the presence of the PE-installed genomic edit.

[0156] FIGs. 70A-70I provide an example of an edited target sequence as an illustration of a step-by-step instruction for designing PEgRNAs and nicking-sgRNAs for prime editing.

FIG. 70A: Step 1. Define the target sequence and the edit. Retrieve the sequence of the target DNA region (~200bp) centered around the location of the desired edit (point mutation, insertion, deletion, or combination thereof). **FIG. 70B: Step 2. Locate target PAMs**. Identify PAMs in proximity to the edit location. Be sure to look for PAMs on both strands. While PAMs close to the edit position are preferred, it is possible to install edits using protospacers and PAMs that place the nick $\geq$ 30 nt from the edit position. **FIG. 70C: Step 3. Locate the nick sites**. For each PAM being considered, identify the corresponding nick site. For Sp Cas9 H840A nickase, cleavage occurs in the PAM-containing strand between the 3rd and 4th bases 5′ to the NGG PAM. All edited nucleotides must exist 3′ of the nick site, so appropriate PAMs must place the nick 5′ to the target edit on the PAM-containing strand. In

the example shown below, there are two possible PAMs. For simplicity, the remaining steps will demonstrate the design of a PEgRNA using PAM 1 only. **FIG. 70D: Step 4. Design the spacer sequence**. The protospacer of Sp Cas9 corresponds to the 20 nucleotides 5′ to the NGG PAM on the PAM-containing strand. Efficient Pol III transcription initiation requires a G to be the first transcribed nucleotide. If the first nucleotide of the protospacer is a G, the spacer sequence for the PEgRNA is simply the protospacer sequence. If the first nucleotide of the protospacer is not a G, the spacer sequence of the PEgRNA is G followed by the protospacer sequence. **FIG. 70E: Step 5. Design a primer binding site (PBS)**. Using the starting allele sequence, identify the DNA primer on the PAM-containing strand. The 3′ end of the DNA primer is the nucleotide just upstream of the nick site (i.e. the 4th base 5′ to the NGG PAM for Sp Cas9). As a general design principle for use with PE2 and PE3, a PEgRNA primer binding site (PBS) containing 12 to 13 nucleotides of complementarity to the DNA primer can be used for sequences that contain ~40-60% GC content. For sequences with low GC content, longer (14- to 15-nt) PBSs should be tested. For sequences with higher GC content, shorter (8- to 11-nt) PBSs should be tested. Optimal PBS sequences should be determined empirically, regardless of GC content. To design a length-*p* PBS sequence, take the reverse complement of the first *p* nucleotides 5′ of the nick site in the PAM-containing strand using the *starting* allele sequence. **FIG. 70F: Step 6. Design an RT template**. The RT template encodes the designed edit and homology to the sequence adjacent to the edit. Optimal RT template lengths vary based on the target site. For short-range edits (positions +1 to +6), it is recommended to test a short (9 to 12 nt), a medium (13 to 16 nt), and a long (17 to 20 nt) RT template. For long-range edits (positions +7 and beyond), it is recommended to use RT templates that extend at least 5 nt (preferably 10 or more nt) past the position of the edit to allow for sufficient 3′ DNA flap homology. For long-range edits, several RT templates should be screened to identify functional designs. For larger insertions and deletions (≥5 nt), incorporation of greater 3′ homology (~20 nt or more) into the RT template is recommended. Editing efficiency is typically impaired when the RT template encodes the synthesis of a G as the last nucleotide in the reverse transcribed DNA product (corresponding to a C in the RT template of the PEgRNA). As many RT templates support efficient prime editing, avoidance of G as the final synthesized nucleotide is recommended when designing RT templates. To design a length-*r* RT template sequence, use the *desired* allele sequence and take the reverse complement of the first *r* nucleotides 3′ of the nick site in the strand that originally contained the PAM. Note that compared to SNP edits, insertion or deletion edits using RT templates of

the same length will not contain identical homology. **FIG. 70G: Step 7. Assemble the full PEgRNA sequence**. Concatenate the PEgRNA components in the following order (5′ to 3′): spacer, scaffold, RT template and PBS. **FIG. 70H: Step 8. Designing nicking-sgRNAs for PE3**. Identify PAMs on the non-edited strand upstream and downstream of the edit. Optimal nicking positions are highly locus-dependent and should be determined empirically. In general, nicks placed 40 to 90 nucleotides 5′ to the position across from the PEgRNA-induced nick lead to higher editing yields and fewer indels. A nicking sgRNA has a spacer sequence that matches the 20-nt protospacer in the *starting* allele, with the addition of a 5′-G if the protospacer does not begin with a G. **FIG. 70I: Step 9. Designing PE3b nicking-sgRNAs**. If a PAM exists in the complementary strand and its corresponding protospacer overlaps with the sequence targeted for editing, this edit could be a candidate for the PE3b system. In the PE3b system, the spacer sequence of the nicking-sgRNA matches the sequence of the desired edited allele, but not the starting allele. The PE3b system operates efficiently when the edited nucleotide(s) falls within the seed region (~10 nt adjacent to the PAM) of the nicking-sgRNA protospacer. This prevents nicking of the complementary strand until after installation of the edited strand, preventing competition between the PEgRNA and the sgRNA for binding the target DNA. PE3b also avoids the generation of simultaneous nicks on both strands, thus reducing indel formation significantly while maintaining high editing efficiency. PE3b sgRNAs should have a spacer sequence that matches the 20-nt protospacer in the *desired* allele, with the addition of a 5′ G if needed.

[0157] FIG. 71A shows the nucleotide sequence of a SpCas9 PEgRNA molecule (top) which terminates at the 3′ end in a "UUU" and does not contain a toeloop element. The lower portion of the figure depicts the same SpCas9 PEgRNA molecule but is further modified to contain a toeloop element having the sequence 5′-"GAAANNNNN"-3′ inserted immediately before the "UUU" 3′ end. The "N" can be any nucleobase.

[0158] FIG. 71B shows the results of Example 18, which demonstrates that the efficiency of prime editing in HEK cells or EMX cells is increased using PEgRNA containing toeloop elements, whereas the percent of indel formation is largely unchanged.

[0159] FIG. 72 depicts alternative PEgRNA configurations that can be used in prime editing. (a) Depicts the PE2:PEgRNA embodiment of prime editing. This embodiment involves a PE2 (a fusion protein comprising a Cas9 and a reverse transcriptase) complexed with a PEgRNA (as also described in FIGs. 1A-1I and/or FIG. 3A-3E). In this embodiment, the template for reverse transcription is incorporated into a 3' extension arm on the sgRNA to

make the PEgRNA, and the DNA polymerase enzyme is a reverse transcriptase (RT) fused directly to Cas9. (b) Depicts the MS2cp-PE2:sgRNA + tPERT embodiment. This embodiment comprises a PE2 fusion (Cas9 + a reverse transcriptase) that is further fused to the MS2 bacteriophage coat protein (MS2cp) to form the MS2cp-PE2 fusion protein. To achieve prime editing, the MS2cp-PE2 fusion protein is complexed with an sgRNA that targets the complex to a specific target site in the DNA. The embodiment then involves the introduction of a *trans* prime editing RNA template ("tPERT"), which operates in place of a PEgRNA by providing a primer binding site (PBS) and an DNA synthesis template on separate molecule, i.e., the tPERT, which is also equipped with a MS2 aptamer (stem loop). The MS2cp protein recruits the tPERT by binding to the MS2 aptamer of the molecule. (c) Depicts alternative designs for PEgRNAs that can be achieved through known methods for chemical synthesis of nucleic acid molecules. For example, chemical synthesis can be used to synthesize a hybrid RNA/DNA PEgRNA molecule for use in prime editing, wherein the extension arm of the hybrid PEgRNA is DNA instead of RNA. In such an embodiment, a DNA-dependent DNA polymerase can be used in place of a reverse transcriptase to synthesize the 3′ DNA flap comprising the desired genetic change that is formed by prime editing. In another embodiment, the extension arm can be synthesized to include a chemical linker that prevents the DNA polymerase (e.g., a reverse transcriptase) from using the sgRNA scaffold or backbone as a template. In still another embodiment, the extension arm may comprise a DNA synthesis template that has the reverse orientation relative to the overall orientation of the PEgRNA molecule. For example, and as shown for a PEgRNA in the 5′-to-3′ orientation and with an extension attached to the 3′ end of the sgRNA scaffold, the DNA synthesis template is orientated in the opposite direction, i.e., the 3′-to-5′ direction. This embodiment may be advantageous for PEgRNA embodiments with extension arms positioned at the 3' end of a gRNA. By reverse the orientation of the extension arm, the DNA synthesis by the polymerase (e.g., reverse transcriptase) will terminate once it reaches the newly orientated 5' of the extension arm and will thus, not risk using the gRNA core as a template.

[0160] FIG. 73 demonstrates prime editing with tPERTs and the MS2 recruitment system (aka MS2 tagging technique). An sgRNA targeting the prime editor protein (PE2) to the target locus is expressed in combination with a tPERT containing a primer binding site (a13-nt or 17-nt PBS), an RT template encoding a His6 tag insertion and a homology arm, and an MS2 aptamer (located at the 5′ or 3′ end of the tPERT molecule). Either prime editor protein

(PE2) or a fusion of the MS2cp to the N-terminus of PE2 was used. Editing was carried out with or without a complementary-strand nicking sgRNA, as in the previously developed PE3 system (designated in the x-axis as labels "PE2+nick" or "PE2", respectively). This is also referred to and defined herein as "second-strand nicking."

[0161] FIG. 74 demonstrates that the MS2 aptamer expression of the reverse transcriptase *in trans* and its recruitment with the MS2 aptamer system. The PEgRNAPEgRNA contains the MS2 RNA aptamer inserted into either one of two sgRNA scaffold hairpins. The wild-type M-MLV reverse transcriptase is expressed as an N-terminal or C-terminal fusion to the MS2 coat protein (MCP). Editing is at the HEK3 site in HEK293T cells.

[0162] FIG. 75 provides a bar graph comparing the efficiency (i.e., "% of total sequencing reads with the specified edit or indels") of PE2, PE2-trunc, PE3, and PE3-trunc over different target sites in various cell lines. The data shows that the prime editors comprising the truncated RT variants were about as efficient as the prime editors comprising the non-truncated RT proteins.

[0163] FIG. 76 demonstrates the editing efficiency of intein-split prime editors fo Example 20. HEK239T cells were transfected with plasmids encoding full-length PE2 or intein-split PE2, PEgRNA and nicking guide RNA. Consensus sequence (most amino-terminal residues of C terminal extein) are indicated. Percent editing at two sites in shown: HEK3 +1 CTT insertion and PRNP +6 G to T. Replicate n=3 independent transfections. See Example 20.

[0164] FIG. 77 demonstrates the editing efficiency of intein-split prime editors fo Example 20. Editing assessed by targeted deep sequencing in bulk cortex and GFP+ subpopulation upon delivery of 5E10vg per SpPE3 half and a small amount 1E10 of nuclear-localized GFP:KASH to P0 mice by ICV injection. Editors and GFP were packaged in AAV9 with EFS promoter. Mice were harvested three weeks post injection and GFP+ nuclei were isolated by flow cytometry. Individual data points are shown, with 1-2 mice per condition analyzed. See Example 20.

[0165] FIG. 78 demonstrates the editing efficiency of intein-split prime editors of Example 20. Specifically, the figures depicts the AAV split-SpPE3 constructs using in Example 20. Co-transduction by AAV particles separately expressing SpPE3-N and SpPE3-C recapitulates PE3 activity. Note N-terminal genome contains a U6-sgRNA cassette expressing the nicking sgRNA, and the C-terminal genome contains a U6-PEgRNA cassette expressing the PEgRNA. See Example 20.

[0166] FIG. 79 shows the editing efficiency of certain optimized linkers as discussed in Example 21. In particular, the data shows the editing efficiency of the PE2 construct with the current linker (noted as PE2 – white box) compared to various versions with the linker replaced with a sequence as indicated at the HEK3, EMX1, FANCF, RNF2 loci for representative PEgRNAs for transition, transversion, insertion, and deletion edits. The replacement linkers are referred to as "1x SGGS", "2x SGGS", "3x SGGS", "1x XTEN", "no linker", "1x Gly", "1x Pro", "1x EAAAK", "2x EAAAK", and "3x EAAAK". The editing efficiency is measured in bar graph format relative to the "control" editing efficiency of PE2. The linker of PE2 is SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127). All editing was done in the context of the PE3 system, i.e., which refers the PE2 editing construct plus the addition of the optimal secondary sgRNA nicking guide. See Example 21.

[0167] FIG. 80. Taking the average fold efficacy relative to PE2 yields the graph shown, indicating that use of a 1x XTEN linker sequence improves editing efficiency by 1.14 fold on average (n=15). See Example 21.

[0168] FIG. 81 depicts the transcription level of PEgRNAs from different promoters, as described in Example 22.

[0169] FIG. 82 As depicted in Example 22, impact of different types of modifications on PEgRNA structure on editing efficiency relative to unmodified PEgRNA.

[0170] FIG. 83 Depicts a PE experiment that targeted editing of the HEK3 gene, specifically targeting the insertion of a 10 nt insertion at position +1 relative to the nick site and using PE3. See Example 22.

[0171] FIG. 84A depicts an exemplary PEgRNA having a spacer, gRNA core, and an extension arm (RT template + primer binding site), which is modified at the 3' end of the PEgRNA with a tRNA molecule, coupled through a UCU linker. The tRNA includes various post-transcriptional modifications. Said modification are not required, however.

[0172] FIG. 84B depicts structure of tRNA that can be used to modify PEgRNA structures. See Example 22. The P1 can be variable in length. The P1 can be extended to help prevent RNAseP processing of the PEgRNA-tRNA fusion.

[0173] FIG. 85 depicts a PE experiment that targeted editing of the FANCF gene, specifically targeting a G-to-T conversion at position +5 relative to the nick site and using PE3 construct. See Example 22.

[0174] FIG. 86 depicts a PE experiment that targeted editing of the HEK3 gene, specifically targeting the insertion of a 71 nt FLAG tag insertion at position +1 relative to the nick site and using PE3 construct. See Example 22.

[0175] FIG. 87 results from a screen in N2A cells where the pegRNA installs 1412Adel, with details about the primer binding site (PBS) length and reverse transcriptase (RT) template length. (Shown with and without indels). See Example 23.

[0176] FIG. 88 results from a screen in N2A cells where the pegRNA installs 1412Adel, with details about the primer binding site (PBS) length and reverse transcriptase (RT) template length. (Shown with and without indels). See Example 23.

[0177] FIG. 89 depicts results of editing at a proxy locus in the β-globin gene and at HEK3 in healthy HSCs, varying the concentration of editor to pegRNA and nicking gRNA. See Example 23.

DEFINITIONS

[0178] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

Antisense strand

[0179] In genetics, the "antisense" strand of a segment within double-stranded DNA is the template strand, and which is considered to run in the 3' to 5' orientation. By contrast, the "sense" strand is the segment within double-stranded DNA that runs from 5' to 3', and which is complementary to the antisense strand of DNA, or template strand, which runs from 3' to 5'. In the case of a DNA segment that encodes a protein, the sense strand is the strand of DNA that has the same sequence as the mRNA, which takes the antisense strand as its template during transcription, and eventually undergoes (typically, not always) translation into a protein. The antisense strand is thus responsible for the RNA that is later translated to protein, while the sense strand possesses a nearly identical makeup to that of the mRNA. Note that for each segment of dsDNA, there will possibly be two sets of sense and antisense,

depending on which direction one reads (since sense and antisense is relative to perspective). It is ultimately the gene product, or mRNA, that dictates which strand of one segment of dsDNA is referred to as sense or antisense.

Bi-specific ligand

[0180] The term "bi-specific ligand" or "bi-specific moiety," as used herein, refers to a ligand that binds to two different ligand-binding domains. In certain embodiments, the ligand is a small molecule compound, or a peptide, or a polypeptide. In other embodiments, ligand-binding domain is a "dimerization domain," which can be install as a peptide tag onto a protein. In various embodiments, two proteins each comprising the same or different dimerization domains can be induced to dimerize through the binding of each dimerization domain to the bi-specific ligand. As used herein, "bi-specific ligands" may be equivalently refer to "chemical inducers of dimerization" or "CIDs".

Cas9

[0181] The term "Cas9" or "Cas9 nuclease" refers to an RNA-guided nuclease comprising a Cas9 domain, or a fragment thereof (*e.g.*, a protein comprising an active or inactive DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A "Cas9 domain" as used herein, is a protein fragment comprising an active or inactive cleavage domain of Cas9 and/or the gRNA binding domain of Cas9. A "Cas9 protein" is a full length Cas9 protein. A Cas9 nuclease is also referred to sometimes as a casn1 nuclease or a CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat)-associated nuclease. CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements, and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 domain. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, *e.g.*, Jinek M., Chylinski K., Fonfara I., Hauer M.,

Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of which are hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see, *e.g.*, "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti *et al.*, J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) *RNA Biology* 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease comprises one or more mutations that partially impair or inactivate the DNA cleavage domain.

[0182] A nuclease-inactivated Cas9 domain may interchangeably be referred to as a "dCas9" protein (for nuclease-"dead" Cas9). Methods for generating a Cas9 domain (or a fragment thereof) having an inactive DNA cleavage domain are known (see, *e.g.*, Jinek *et al.*, *Science*. 337:816-821(2012); Qi *et al.*, "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression" (2013) *Cell*. 28;152(5):1173-83, the entire contents of each of which are incorporated herein by reference). For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D10A and H840A completely inactivate the nuclease activity of *S. pyogenes* Cas9

(Jinek *et al.*, *Science*. 337:816-821(2012); Qi *et al.*, *Cell*. 28;152(5):1173-83 (2013)). In some embodiments, proteins comprising fragments of Cas9 are provided. For example, in some embodiments, a protein comprises one of two Cas9 domains: (1) the gRNA binding domain of Cas9; or (2) the DNA cleavage domain of Cas9. In some embodiments, proteins comprising Cas9 or fragments thereof are referred to as "Cas9 variants." A Cas9 variant shares homology to Cas9, or a fragment thereof. For example, a Cas9 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, at least about 99.8% identical, or at least about 99.9% identical to wild type Cas9 (e.g., SpCas9 of SEQ ID NO: 18). In some embodiments, the Cas9 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more amino acid changes compared to wild type Cas9 (e.g., SpCas9 of SEQ ID NO: 18). In some embodiments, the Cas9 variant comprises a fragment of SEQ ID NO: 18 Cas9 (*e.g.*, a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type Cas9 (e.g., SpCas9 of SEQ ID NO: 18). In some embodiments, the fragment is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type Cas9 (e.g., SpCas9 of SEQ ID NO: 18).

cDNA

[0183] The term "cDNA" refers to a strand of DNA copied from an RNA template. cDNA is complementary to the RNA template.

Circular permutant

[0184] As used herein, the term "circular permutant" refers to a protein or polypeptide (e.g., a Cas9) comprising a circular permutation, which is change in the protein's structural configuration involving a change in order of amino acids appearing in the protein's amino acid sequence. In other words, circular permutants are proteins that have altered N- and C-

termini as compared to a wild-type counterpart, e.g., the wild-type C-terminal half of a protein becomes the new N-terminal half. Circular permutation (or CP) is essentially the topological rearrangement of a protein's primary sequence, connecting its N- and C-terminus, often with a peptide linker, while concurrently splitting its sequence at a different position to create new, adjacent N- and C-termini. The result is a protein structure with different connectivity, but which often can have the same overall similar three-dimensional (3D) shape, and possibly include improved or altered characteristics, including, reduced proteolytic susceptibility, improved catalytic activity, altered substrate or ligand binding, and/or improved thermostability. Circular permutant proteins can occur in nature (e.g., concanavalin A and lectin). In addition, circular permutation can occur as a result of posttranslational modifications or may be engineered using recombinant techniques.

Circularly permuted Cas9

[0185] The term "circularly permuted Cas9" refers to any Cas9 protein, or variant thereof, that has been occurs as a circular permutant, whereby its N- and C-termini have been topically rearranged. Such circularly permuted Cas9 proteins ("CP-Cas9"), or variants thereof, retain the ability to bind DNA when complexed with a guide RNA (gRNA). See, Oakes et al., "Protein Engineering of Cas9 for enhanced function," *Methods Enzymol*, 2014, 546: 491–511 and Oakes et al., "CRISPR-Cas9 Circular Permutants as Programmable Scaffolds for Genome Modification," *Cell*, January 10, 2019, 176: 254-267, each of are incorporated herein by reference. The instant disclosure contemplates any previously known CP-Cas9 or use a new CP-Cas9 so long as the resulting circularly permuted protein retains the ability to bind DNA when complexed with a guide RNA (gRNA). Exemplary CP-Cas9 proteins are SEQ ID NOs: 77-86.

CRISPR

[0186] CRISPR is a family of DNA sequences (i.e., CRISPR clusters) in bacteria and archaea that represent snippets of prior infections by a virus that have invaded the prokaryote. The snippets of DNA are used by the prokaryotic cell to detect and destroy DNA from subsequent attacks by similar viruses and effectively compose, along with an array of CRISPR-associated proteins (including Cas9 and homologs thereof) and CRISPR-associated RNA, a prokaryotic immune defense system. In nature, CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In certain types of CRISPR systems (e.g., type II CRISPR systems), correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a

guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the RNA. Specifically, the target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species – the guide RNA. See, *e.g.*, Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. CRISPR biology, as well as Cas9 nuclease sequences and structures are well known to those of skill in the art (see, *e.g.*, "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti *et al.*, J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference.

[0187] In certain types of CRISPR systems (e.g., type II CRISPR systems), correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc), and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular nucleic acid target complementary to the RNA. Specifically, the

target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gRNA") can be engineered so as to incorporate embodiments of both the crRNA and tracrRNA into a single RNA species—the guide RNA.

[0188] In general, a "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus. The tracrRNA of the system is complementary (fully or partially) to the tracr mate sequence present on the guide RNA.

DNA synthesis template

[0189] As used herein, the term "DNA synthesis template" refers to the region or portion of the extension arm of a PEgRNA that is utilized as a template strand by a polymerase of a prime editor to encode a 3′ single-strand DNA flap that contains the desired edit and which then, through the mechanism of prime editing, replaces the corresponding endogenous strand of DNA at the target site. In various embodiments, the DNA synthesis template is shown in FIG. 3A (in the context of a PEgRNA comprising a 5′ extension arm), FIG. 3B (in the context of a PEgRNA comprising a 3′ extension arm), FIG. 3C (in the context of an internal extension arm), FIG. 3D (in the context of a 3′ extension arm), and FIG. 3E (in the context of a 5′ extension arm). The extension arm, including the DNA synthesis template, may be comprised of DNA or RNA. In the case of RNA, the polymerase of the prime editor can be an RNA-dependent DNA polymerase (e.g., a reverse transcriptase). In the case of DNA, the polymerase of the prime editor can be a DNA-dependent DNA polymerase. In various embodiments (e.g., as depicted in FIGs. 3D-3E), the DNA synthesis template (4) may comprise the "edit template" and the "homology arm", and all or a portion of the optional 5' end modifier region, e2. That is, depending on the nature of the e2 region (e.g., whether it includes a hairpin, toeloop, or stem/loop secondary structure), the polymerase may encode none, some, or all of the e2 region, as well. Said another way, in the case of a 3′ extension arm, the DNA synthesis template (3) can include the portion of the extension arm (3) that

spans from the 5′ end of the primer binding site (PBS) to 3′ end of the gRNA core that may operate as a template for the synthesis of a single-strand of DNA by a polymerase (e.g., a reverse transcriptase). In the case of a 5′ extension arm, the DNA synthesis template (3) can include the portion of the extension arm (3) that spans from the 5′ end of the PEgRNA molecule to the 3′ end of the edit template. Preferably, the DNA synthesis template excludes the primer binding site (PBS) of PEgRNAs either having a 3′ extension arm or a 5′ extension arm. Certain embodiments described here (e.g, FIG. 71A) refer to an "an RT template," which is inclusive of the edit template and the homology arm, i.e., the sequence of the PEgRNA extension arm which is actually used as a template during DNA synthesis. The term "RT template" is equivalent to the term "DNA synthesis template."

[0190] In the case of *trans* prime editing (e.g., FIG. 3G and FIG. 3H), the primer binding site (PBS) and the DNA synthesis template can be engineered into a separate molecule referred to as a *trans* prime editor RNA template (tPERT).

Dimerization domain

[0191] The term "dimerization domain" refers to a ligand-binding domain that binds to a binding moiety of a bi-specific ligand. A "first" dimerization domain binds to a first binding moiety of a bi-specific ligand and a "second" dimerization domain binds to a second binding moiety of the same bi-specific ligand. When the first dimerization domain is fused to a first protein (e.g., via PE, as discussed herein) and the second dimerization domain (e.g., via PE, as discussed herein) is fused to a second protein, the first and second protein dimerize in the presence of a bi-specific ligand, wherein the bi-specific ligand has at least one moiety that binds to the first dimerization domain and at least another moiety that binds to the second dimerization domain.

Downstream

[0192] As used herein, the terms "upstream" and "downstream" are terms of relativity that define the linear position of at least two elements located in a nucleic acid molecule (whether single or double-stranded) that is orientated in a 5′-to-3′ direction. In particular, a first element is upstream of a second element in a nucleic acid molecule where the first element is positioned somewhere that is 5' to the second element. For example, a SNP is upstream of a Cas9-induced nick site if the SNP is on the 5' side of the nick site. Conversely, a first element is downstream of a second element in a nucleic acid molecule where the first element is positioned somewhere that is 3' to the second element. For example, a SNP is downstream of a Cas9-induced nick site if the SNP is on the 3' side of the nick site. The nucleic acid

molecule can be a DNA (double or single stranded). RNA (double or single stranded), or a hybrid of DNA and RNA. The analysis is the same for single strand nucleic acid molecule and a double strand molecule since the terms upstream and downstream are in reference to only a single strand of a nucleic acid molecule, except that one needs to select which strand of the double stranded molecule is being considered. Often, the strand of a double stranded DNA which can be used to determine the positional relativity of at least two elements is the "sense" or "coding" strand. In genetics, a "sense" strand is the segment within double-stranded DNA that runs from 5' to 3', and which is complementary to the antisense strand of DNA, or template strand, which runs from 3' to 5'. Thus, as an example, a SNP nucleobase is "downstream" of a promoter sequence in a genomic DNA (which is double-stranded) if the SNP nucleobase is on the 3' side of the promoter on the sense or coding strand.

Edit template

[0193] The term "edit template" refers to a portion of the extension arm that encodes the desired edit in the single strand 3′ DNA flap that is synthesized by the polymerase, e.g., a DNA-dependent DNA polymerase, RNA-dependent DNA polymerase (e.g., a reverse transcriptase). Certain embodiments described here (e.g., FIG. 71A) refer to "an RT template," which refers to both the edit template and the homology arm together, i.e., the sequence of the PEgRNA extension arm which is actually used as a template during DNA synthesis. The term "RT edit template" is also equivalent to the term "DNA synthesis template," but wherein the RT edit template reflects the use of a prime editor having a polymerase that is a reverse transcriptase, and wherein the DNA synthesis template reflects more broadly the use of a prime editor having any polymerase.

Effective amount

[0194] The term "effective amount," as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a desired biological response. For example, in some embodiments, an effective amount of a prime editor (PE) may refer to the amount of the editor that is sufficient to edit a target site nucleotide sequence, e.g., a genome. In some embodiments, an effective amount of a prime editor (PE) provided herein, e.g., of a fusion protein comprising a nickase Cas9 domain and a reverse transcriptase may refer to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the fusion protein. As will be appreciated by the skilled artisan, the effective amount of an agent, e.g., a fusion protein, a nuclease, a hybrid protein, a protein dimer, a complex of a protein (or protein dimer) and a polynucleotide, or a polynucleotide,

may vary depending on various factors as, for example, on the desired biological response, e.g., on the specific allele, genome, or target site to be edited, on the cell or tissue being targeted, and on the agent being used.

Error-prone reverse transcriptase

[0195] As used herein, the term "error-prone" reverse transcriptase (or more broadly, any polymerase) refers to a reverse transcriptase (or more broadly, any polymerase) that occurs naturally or which has been derived from another reverse transcriptase (e.g., a wild type M-MLV reverse transcriptase) which has an error rate that is less than the error rate of wild type M-MLV reverse transcriptase. The error rate of wild type M-MLV reverse transcriptase is reported to be in the range of one error in 15,000 (higher) to 27,000 (lower). An error rate of 1 in 15,000 corresponds with an error rate of 6.7×10^{-5}. An error rate of 1 in 27,000 corresponds with an error rate of 3.7×10^{-5}. See Boutabout et al. (2001) "DNA synthesis fidelity by the reverse transcriptase of the yeast retrotransposon Ty1," *Nucleic Acids Res* 29(11):2217–2222, which is incorporated herein by reference. Thus, for purposes of this application, the term "error prone" refers to those RT that have an error rate that is greater than one error in 15,000 nucleobase incorporation (6.7×10^{-5} or higher), e.g., 1 error in 14,000 nucleobases (7.14×10^{-5} or higher), 1 error in 13,000 nucleobases or fewer (7.7×10^{-5} or higher), 1 error in 12,000 nucleobases or fewer (7.7×10^{-5} or higher), 1 error in 11,000 nucleobases or fewer (9.1×10^{-5} or higher), 1 error in 10,000 nucleobases or fewer (1×10^{-4} or 0.0001 or higher), 1 error in 9,000 nucleobases or fewer (0.00011 or higher), 1 error in 8,000 nucleobases or fewer (0.00013 or higher) 1 error in 7,000 nucleobases or fewer (0.00014 or higher), 1 error in 6,000 nucleobases or fewer (0.00016 or higher), 1 error in 5,000 nucleobases or fewer (0.0002 or higher), 1 error in 4,000 nucleobases or fewer (0.00025 or higher), 1 error in 3,000 nucleobases or fewer (0.00033 or higher), 1 error in 2,000 nucleobase or fewer (0.00050 or higher), or 1 error in 1,000 nucleobases or fewer (0.001 or higher), or 1 error in 500 nucleobases or fewer (0.002 or higher), or 1 error in 250 nucleobases or fewer (0.004 or higher).

Extein

[0196] The term "extein," as used herein, refers to an polypeptide sequence that is flanked by an intein and is ligated to another extein during the process of protein splicing to form a mature, spliced protein. Typically, an intein is flanked by two extein sequences that are ligated together when the intein catalyzes its own excision. Exteins, accordingly, are the protein analog to exons found in mRNA. For example, a polypeptide comprising an intein

may be of the structure extein(N) – intein – extein(C). After excision of the intein and splicing of the two exteins, the resulting structures are extein(N) – extein(C) and a free intein. In various configurations, the exteins may be separate proteins (e.g., half of a Cas9 or PE fusion protein), each fused to a split-intein, wherein the excision of the split inteins causes the splicing together of the extein sequences.

Extension arm

[0197] The term "extension arm" refers to a nucleotide sequence component of a PEgRNA which provides several functions, including a primer binding site and an edit template for reverse transcriptase. In some embodiments, e.g., FIG. 3D, the extension arm is located at the 3′ end of the guide RNA. In other embodiments, e.g., FIG. 3E, the extension arm is located at the 5′ end of the guide RNA. In some embodiments, the extension arm also includes a homology arm. In various embodiments, the extension arm comprises the following components in a 5′ to 3′ direction: the homology arm, the edit template, and the primer binding site. Since polymerization activity of the reverse transcriptase is in the 5′ to 3′ direction, the preferred arrangement of the homology arm, edit template, and primer binding site is in the 5′ to 3′ direction such that the reverse transcriptase, once primed by an annealed primer sequence, polymerases a single strand of DNA using the edit template as a complementary template strand. Further details, such as the length of the extension arm, are described elsewhere herein.

[0198] The extension arm may also be described as comprising generally two regions: a primer binding site (PBS) and a DNA synthesis template, as shown in FIG. 3G (top), for instance. The primer binding site binds to the primer sequence that is formed from the endogenous DNA strand of the target site when it becomes nicked by the prime editor complex, thereby exposing a 3′ end on the endogenous nicked strand. As explained herein, the binding of the primer sequence to the primer binding site on the extension arm of the PEgRNA creates a duplex region with an exposed 3′ end (i.e., the 3′ of the primer sequence), which then provides a substrate for a polymerase to begin polymerizing a single strand of DNA from the exposed 3′ end along the length of the DNA synthesis template. The sequence of the single strand DNA product is the complement of the DNA synthesis template. Polymerization continues towards the 5′ of the DNA synthesis template (or extension arm) until polymerization terminates. Thus, the DNA synthesis template represents the portion of the extension arm that is encoded into a single strand DNA product (i.e., the 3′ single strand DNA flap containing the desired genetic edit information) by the polymerase of the prime

editor complex and which ultimately replaces the corresponding endogenous DNA strand of the target site that sits immediate downstream of the PE-induced nick site. Without being bound by theory, polymerization of the DNA synthesis template continues towards the 5′ end of the extension arm until a termination event. Polymerization may terminate in a variety of ways, including, but not limited to (a) reaching a 5′ terminus of the PEgRNA (e.g., in the case of the 5′ extension arm wherein the DNA polymerase simply runs out of template), (b) reaching an impassable RNA secondary structure (e.g., hairpin or stem/loop), or (c) reaching a replication termination signal, e.g., a specific nucleotide sequence that blocks or inhibits the polymerase, or a nucleic acid topological signal, such as, supercoiled DNA or RNA.

Flap endonuclease (e.g., FEN1)

[0199] As used herein, the term "flap endonuclease" refers to an enzyme that catalyzes the removal of 5′ single strand DNA flaps. These are naturally occurring enzymes that process the removal of 5′ flaps formed during cellular processes, including DNA replication. The prime editing methods herein described may utilize endogenously supplied flap endonucleases or those provided *in trans* to remove the 5′ flap of endogenous DNA formed at the target site during prime editing. Flap endonucleases are known in the art and can be found described in Patel et al., "Flap endonucleases pass 5′-flaps through a flexible arch using a disorder-thread-order mechanism to confer specificity for free 5′-ends," *Nucleic Acids Research*, 2012, 40(10): 4507-4519, Tsutakawa et al., "Human flap endonuclease structures, DNA double-base flipping, and a unified understanding of the FEN1 superfamily," *Cell*, 2011, 145(2): 198-211, and Balakrishnan et al., "Flap Endonuclease 1," *Annu Rev Biochem*, 2013, Vol 82: 119-138 (each of which are incorporated herein by reference). An exemplary flap endonuclease is FEN1, which can be represented by the following amino acid sequence:

DESCRIPTION	SEQUENCE	SEQ ID NO:
FEN1 WILD TYPE	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSI YQFLIAVRQGGDVLQNEEGETTSHLMGMFYRTIRMME NGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQ AQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI PYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGS PVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFVDL CILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLDPN KYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEPNE EELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLD DFFKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFKR GK	SEQ ID NO: 7

Functional equivalent

[0200] The term "functional equivalent" refers to a second biomolecule that is equivalent in function, but not necessarily equivalent in structure to a first biomolecule. For example, a "Cas9 equivalent" refers to a protein that has the same or substantially the same functions as Cas9, but not necessarily the same amino acid sequence. In the context of the disclosure, the specification refers throughout to "a protein X, or a functional equivalent thereof." In this context, a "functional equivalent" of protein X embraces any homolog, paralog, fragment, naturally occurring, engineered, mutated, or synthetic version of protein X which bears an equivalent function.

Fusion protein

[0201] The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein may be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an "amino-terminal fusion protein" or a "carboxy-terminal fusion protein," respectively. A protein may comprise different domains, for example, a nucleic acid binding domain (*e.g.*, the gRNA binding domain of Cas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain or a catalytic domain of a nucleic-acid editing protein. Another example includes a Cas9 or equivalent thereof to a reverse transcriptase. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4^{th} ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

Gene of interest (GOI)

[0202] The term "gene of interest" or "GOI" refers to a gene that encodes a biomolecule of interest (e.g., a protein or an RNA molecule). A protein of interest can include any intracellular protein, membrane protein, or extracellular protein, e.g., a nuclear protein, transcription factor, nuclear membrane transporter, intracellular organelle associated protein, a membrane receptor, a catalytic protein, and enzyme, a therapeutic protein, a membrane protein, a membrane transport protein, a signal transduction protein, or an immunological protein (e.g., an IgG or other antibody protein), etc. The gene of interest may also encode an

RNA molecule, including, but not limited to, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), antisense RNA, guide RNA, microRNA (miRNA), small interfering RNA (siRNA), and cell-free RNA (cfRNA).

Guide RNA ("gRNA")

[0203] As used herein, the term "guide RNA" is a particular type of guide nucleic acid which is mostly commonly associated with a Cas protein of a CRISPR-Cas9 and which associates with Cas9, directing the Cas9 protein to a specific sequence in a DNA molecule that includes complementarity to protospacer sequence of the guide RNA. However, this term also embraces the equivalent guide nucleic acid molecules that associate with Cas9 equivalents, homologs, orthologs, or paralogs, whether naturally occurring or non-naturally occurring (e.g., engineered or recombinant), and which otherwise program the Cas9 equivalent to localize to a specific target nucleotide sequence. The Cas9 equivalents may include other napDNAbp from any type of CRISPR system (e.g., type II, V, VI), including Cpf1 (a type-V CRISPR-Cas systems), C2c1 (a type V CRISPR-Cas system), C2c2 (a type VI CRISPR-Cas system) and C2c3 (a type V CRISPR-Cas system). Further Cas-equivalents are described in Makarova et al., "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector," Science 2016; 353(6299), the contents of which are incorporated herein by reference. Exemplary sequences are and structures of guide RNAs are provided herein. In addition, methods for designing appropriate guide RNA sequences are provided herein. As used herein, the "guide RNA" may also be referred to as a "traditional guide RNA" to contrast it with the modified forms of guide RNA termed "prime editing guide RNAs" (or "PEgRNAs") which have been invented for the prime editing methods and composition disclosed herein.

[0204] Guide RNAs or PEgRNAs may comprise various structural elements that include, but are not limited to:

[0205] Spacer sequence – the sequence in the guide RNA or PEgRNA (having about 20 nts in length) which binds to the protospacer in the target DNA.

[0206] gRNA core (or gRNA scaffold or backbone sequence) - refers to the sequence within the gRNA that is responsible for Cas9 binding, it does not include the 20 bp spacer/targeting sequence that is used to guide Cas9 to target DNA.

[0207] Extension arm – a single strand extension at the 3′ end or the 5′ end of the PEgRNA which comprises a primer binding site and a DNA synthesis template sequence that encodes via a polymerase (e.g., a reverse transcriptase) a single stranded DNA flap containing the

genetic change of interest, which then integrates into the endogenous DNA by replacing the corresponding endogenous strand, thereby installing the desired genetic change.

[0208] Transcription terminator – the guide RNA or PEgRNA may comprise a transcriptional termination sequence at the 3′ of the molecule.

Homology arm

[0209] The term "homology arm" refers to a portion of the extension arm that encodes a portion of the resulting reverse transcriptase-encoded single strand DNA flap that is to be integrated into the target DNA site by replacing the endogenous strand. The portion of the single strand DNA flap encoded by the homology arm is complementary to the non-edited strand of the target DNA sequence, which facilitates the displacement of the endogenous strand and annealing of the single strand DNA flap in its place, thereby installing the edit. This component is further defined elsewhere. The homology arm is part of the DNA synthesis template since it is by definition encoded by the polymerase of the prime editors described herein.

Host cell

[0210] The term "host cell," as used herein, refers to a cell that can host, replicate, and express a vector described herein, e.g., a vector comprising a nucleic acid molecule encoding a fusion protein comprising a Cas9 or Cas9 equivalent and a reverse transcriptase.

Inteins

[0211] As used herein, the term "intein" refers to auto-processing polypeptide domains found in organisms from all domains of life. An intein (*int*ervening prot*ein*) carries out a unique auto-processing event known as protein splicing in which it excises itself out from a larger precursor polypeptide through the cleavage of two peptide bonds and, in the process, ligates the flanking extein (external protein) sequences through the formation of a new peptide bond. This rearrangement occurs post-translationally (or possibly co-translationally), as intein genes are found embedded in frame within other protein-coding genes. Furthermore, intein-mediated protein splicing is spontaneous; it requires no external factor or energy source, only the folding of the intein domain. This process is also known as *cis*-protein splicing, as opposed to the natural process of *trans*-protein splicing with "split inteins." Inteins are the protein equivalent of the self-splicing RNA introns (see Perler et al., Nucleic Acids Res. 22:1125-1127 (1994)), which catalyze their own excision from a precursor protein with the concomitant fusion of the flanking protein sequences, known as exteins (reviewed in Perler et

al., Curr. Opin. Chem. Biol. 1:292-299 (1997); Perler, F. B. Cell 92(1):1-4 (1998); Xu et al., EMBO J. 15(19):5146-5153 (1996)).

[0212] As used herein, the term "protein splicing" refers to a process in which an interior region of a precursor protein (an intein) is excised and the flanking regions of the protein (exteins) are ligated to form the mature protein. This natural process has been observed in numerous proteins from both prokaryotes and eukaryotes (Perler, F. B., Xu, M. Q., Paulus, H. Current Opinion in Chemical Biology 1997, 1, 292-299; Perler, F. B. Nucleic Acids Research 1999, 27, 346-347). The intein unit contains the necessary components needed to catalyze protein splicing and often contains an endonuclease domain that participates in intein mobility (Perler, F. B., Davis, E. O., Dean, G. E., Gimble, F. S., Jack, W. E., Neff, N., Noren, C. J., Thomer, J., Belfort, M. Nucleic Acids Research 1994, 22, 1127-1127). The resulting proteins are linked, however, not expressed as separate proteins. Protein splicing may also be conducted in *trans* with split inteins expressed on separate polypeptides spontaneously combine to form a single intein which then undergoes the protein splicing process to join to separate proteins.

[0213] The elucidation of the mechanism of protein splicing has led to a number of intein-based applications (Comb, et al., U.S. Pat. No. 5,496,714; Comb, et al., U.S. Pat. No. 5,834,247; Camarero and Muir, J. Amer. Chem. Soc., 121:5597-5598 (1999); Chong, et al., Gene, 192:271-281 (1997), Chong, et al., Nucleic Acids Res., 26:5109-5115 (1998); Chong, et al., J. Biol. Chem., 273:10567-10577 (1998); Cotton, et al. J. Am. Chem. Soc., 121:1100-1101 (1999); Evans, et al., J. Biol. Chem., 274:18359-18363 (1999); Evans, et al., J. Biol. Chem., 274:3923-3926 (1999); Evans, et al., Protein Sci., 7:2256-2264 (1998); Evans, et al., J. Biol. Chem., 275:9091-9094 (2000); Iwai and Pluckthun, FEBS Lett. 459:166-172 (1999); Mathys, et al., Gene, 231:1-13 (1999); Mills, et al., Proc. Natl. Acad. Sci. USA 95:3543-3548 (1998); Muir, et al., Proc. Natl. Acad. Sci. USA 95:6705-6710 (1998); Otomo, et al., Biochemistry 38:16040-16044 (1999); Otomo, et al., J. Biolmol. NMR 14:105-114 (1999); Scott, et al., Proc. Natl. Acad. Sci. USA 96:13638-13643 (1999); Severinov and Muir, J. Biol. Chem., 273:16205-16209 (1998); Shingledecker, et al., Gene, 207:187-195 (1998); Southworth, et al., EMBO J. 17:918-926 (1998); Southworth, et al., Biotechniques, 27:110-120 (1999); Wood, et al., Nat. Biotechnol., 17:889-892 (1999); Wu, et al., Proc. Natl. Acad. Sci. USA 95:9226-9231 (1998a); Wu, et al., Biochim Biophys Acta 1387:422-432 (1998b); Xu, et al., Proc. Natl. Acad. Sci. USA 96:388-393 (1999); Yamazaki, et al., J. Am. Chem. Soc., 120:5591-5592 (1998)). Each reference is incorporated herein by reference.

Ligand-dependent intein

[0214] The term "ligand-dependent intein," as used herein refers to an intein that comprises a ligand-binding domain. Typically, the ligand-binding domain is inserted into the amino acid sequence of the intein, resulting in a structure intein (N) – ligand-binding domain – intein (C). Typically, ligand-dependent inteins exhibit no or only minimal protein splicing activity in the absence of an appropriate ligand, and a marked increase of protein splicing activity in the presence of the ligand. In some embodiments, the ligand-dependent intein does not exhibit observable splicing activity in the absence of ligand but does exhibit splicing activity in the presence of the ligand. In some embodiments, the ligand-dependent intein exhibits an observable protein splicing activity in the absence of the ligand, and a protein splicing activity in the presence of an appropriate ligand that is at least 5 times, at least 10 times, at least 50 times, at least 100 times, at least 150 times, at least 200 times, at least 250 times, at least 500 times, at least 1000 times, at least 1500 times, at least 2000 times, at least 2500 times, at least 5000 times, at least 10000 times, at least 20000 times, at least 25000 times, at least 50000 times, at least 100000 times, at least 500000 times, or at least 1000000 times greater than the activity observed in the absence of the ligand. In some embodiments, the increase in activity is dose dependent over at least 1 order of magnitude, at least 2 orders of magnitude, at least 3 orders of magnitude, at least 4 orders of magnitude, or at least 5 orders of magnitude, allowing for fine-tuning of intein activity by adjusting the concentration of the ligand. Suitable ligand-dependent inteins are known in the art, and in include those provided below and those described in published U.S. Patent Application U.S. 2014/0065711 A1; Mootz *et al.*, "Protein splicing triggered by a small molecule." *J. Am. Chem. Soc.* 2002; **124**, 9044–9045; Mootz *et al.*, "Conditional protein splicing: a new tool to control protein structure and function *in vitro* and *in vivo*." *J. Am. Chem. Soc.* 2003; **125**, 10561–10569; Buskirk *et al.*, *Proc. Natl. Acad. Sci. USA.* 2004; **101**, 10505-10510); Skretas & Wood, "Regulation of protein activity with small-molecule-controlled inteins." *Protein Sci.* 2005; **14**, 523-532; Schwartz, *et al.,* "Post-translational enzyme activation in an animal via optimized conditional protein splicing." *Nat. Chem. Biol.* 2007; **3**, 50-54; Peck *et al.*, *Chem. Biol.* 2011; **18** (5), 619-630; the entire contents of each are hereby incorporated by reference. Exemplary sequences are as follows:

NAME	SEQUENCE OF LIGAND-DEPENDENT INTEIN

2-4 INTEIN:	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 8)
3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDR VAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEIL MIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATS SRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRAL DKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHL YSMKYTNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDK FLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHN C (SEQ ID NO: 9)
30R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 10)
30R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 11)
30R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL

	DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 12)
37R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYNPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC ((SEQ ID NO: 13)
37R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 14)
37R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 15)

Linker

[0215] The term "linker," as used herein, refers to a molecule linking two other molecules or moieties. The linker can be an amino acid sequence in the case of a linker joining two fusion proteins. For example, a Cas9 can be fused to a reverse transcriptase by an amino acid linker sequence. The linker can also be a nucleotide sequence in the case of joining two nucleotide sequences together. For example, in the instant case, the traditional guide RNA is linked via a spacer or linker nucleotide sequence to the RNA extension of a prime editing guide RNA which may comprise a RT template sequence and an RT primer binding site. In other embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-100 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-

45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated.

Isolated

[0216] "Isolated" means altered or removed from the natural state. For example, a nucleic 20 acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

[0217] In some embodiments, a gene of interest is encoded by an isolated nucleic acid. As used herein, the term "isolated," refers to the characteristic of a material as provided herein being removed from its original or native environment (e.g., the natural environment if it is naturally occurring). Therefore, a naturally-occurring polynucleotide or protein or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated by human intervention from some or all of the coexisting materials in the natural system, is isolated. An artificial or engineered material, for example, a non-naturally occurring nucleic acid construct, such as the expression constructs and vectors described herein, are, accordingly, also referred to as isolated. A material does not have to be purified in order to be isolated. Accordingly, a material may be part of a vector and/or part of a composition, and still be isolated in that such vector or composition is not part of the environment in which the material is found in nature.

MS2 tagging technique

[0218] In various embodiments (e.g., as depicted in the embodiments of FIGs. 72-73 and in Example 19), the term "MS2 tagging technique" refers to the combination of an "RNA-protein interaction domain" (aka "RNA-protein recruitment domain or protein") paired up with an RNA-binding protein that specifically recognizes and binds to the RNA-protein interaction domain, e.g., a specific hairpin structure. These types of systems can be leveraged to recruit a variety of functionalities to a prime editor complex that is bound to a target site. The MS2 tagging technique is based on the natural interaction of the MS2 bacteriophage coat protein ("MCP" or "MS2cp") with a stem-loop or hairpin structure present in the genome of the phage, i.e., the "MS2 hairpin." In the case of prime editing, the MS2 tagging technique comprises introducing the MS2 hairpin into a desired RNA molecule involved in prime editing (e.g., a PEgRNA or a tPERT), which then constitutes a specific interactable binding target for an RNA-binding protein that recognizes and binds to that structure. In the case of

the MS2 hairpin, it is recognized and bound by the MS2 bacteriophage coat protein (MCP). And, if MCP is fused to another protein (e.g., a reverse transcriptase or other DNA polymerase), then the MS2 hairpin may be used to "recruit" that other protein *in trans* to the target site occupied by the prime editing complex.

[0219] The prime editors described herein may incorporate as an aspect any known RNA-protein interaction domain to recruit or "co-localize" specific functions of interest to a prime editor complex. A review of other modular RNA-protein interaction domains are described in the art, for example, in Johansson et al., "RNA recognition by the MS2 phage coat protein," *Sem Virol.*, 1997, Vol. 8(3): 176-185; Delebecque et al., "Organization of intracellular reactions with rationally designed RNA assemblies," *Science*, 2011, Vol. 333: 470-474; Mali et al., "Cas9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.*, 2013, Vol.31: 833-838; and Zalatan et al., "Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds," *Cell*, 2015, Vol.160: 339-350, each of which are incorporated herein by reference in their entireties. Other systems include the PP7 hairpin, which specifically recruits the PCP protein, and the "com" hairpin, which specifically recruits the Com protein. See Zalatan et al.

[0220] The nucleotide sequence of the MS2 hairpin (or equivalently referred to as the "MS2 aptamer") is: GCCAACATGAGGATCACCCATGTCTGCAGGGCC (SEQ ID NO: 763).

[0221] The amino acid sequence of the MCP or MS2cp is: GSASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQ NRKYTIKVEVPKVATQTVGGEELPVAGWRSYLNMELTIPIFATNSDCELIVKAMQGL LKDGNPIPSAIAANSGIY (SEQ ID NO: 764).

[0222] The MS2 hairpin (or "MS2 aptamer") may also be referred to as a type of "RNA effector recruitment domain"(or equivalently as "RNA-binding protein recruitment domain" or simply as "recruitment domain") since it is a physical structure (e.g., a hairpin) that is installed into a PEgRNA or tPERT that effectively recruits other effector functions (e.g., RNA-binding proteins having various functions, such as DNA polymerases or other DNA-modifying enzymes) to the PEgRNA or rPERT that is so modified, and thus, co-localizing effector functions *in trans* to the prime editing machinery. This application is not intended to be limited in any way to any particular RNA effector recruitment domains and may include any available such domain, including the MS2 hairpin. Example 19 and FIG. 72(b) depicts the use of the MS2 aptamer joined to a DNA synthesis domain (i.e., the tPERT molecule) and

a prime editor that comprises an MS2cp protein fused to a PE2 to cause the co-localization of the prime editor complex (MS2cp-PE2:sgRNA complex) bound to the target DNA site and the DNA synthesis domain of the tPERT molecule to effectuate the

napDNAbp

[0223] As used herein, the term "nucleic acid programmable DNA binding protein" or "napDNAbp," of which Cas9 is an example, refer to a proteins which use RNA:DNA hybridization to target and bind to specific sequences in a DNA molecule. Each napDNAbp is associated with at least one guide nucleic acid (e.g., guide RNA), which localizes the napDNAbp to a DNA sequence that comprises a DNA strand (i.e., a target strand) that is complementary to the guide nucleic acid, or a portion thereof (e.g., the protospacer of a guide RNA). In other words, the guide nucleic-acid "programs" the napDNAbp (e.g., Cas9 or equivalent) to localize and bind to a complementary sequence.

[0224] Without being bound by theory, the binding mechanism of a napDNAbp – guide RNA complex, in general, includes the step of forming an R-loop whereby the napDNAbp induces the unwinding of a double-strand DNA target, thereby separating the strands in the region bound by the napDNAbp. The guide RNA protospacer then hybridizes to the "target strand." This displaces a "non-target strand" that is complementary to the target strand, which forms the single strand region of the R-loop. In some embodiments, the napDNAbp includes one or more nuclease activities, which then cut the DNA leaving various types of lesions. For example, the napDNAbp may comprises a nuclease activity that cuts the non-target strand at a first location, and/ or cuts the target strand at a second location. Depending on the nuclease activity, the target DNA can be cut to form a "double-stranded break" whereby both strands are cut. In other embodiments, the target DNA can be cut at only a single site, i.e., the DNA is "nicked" on one strand. Exemplary napDNAbp with different nuclease activities include "Cas9 nickase" ("nCas9") and a deactivated Cas9 having no nuclease activities ("dead Cas9" or "dCas9"). Exemplary sequences for these and other napDNAbp are provided herein.

Nickase

[0225] The term "nickase" refers to a Cas9 with one of the two nuclease domains inactivated. This enzyme is capable of cleaving only one strand of a target DNA.

Nuclear localization sequence (NLS)

[0226] The term "nuclear localization sequence" or "NLS" refers to an amino acid sequence that promotes import of a protein into the cell nucleus, for example, by nuclear transport. Nuclear localization sequences are known in the art and would be apparent to the skilled

artisan. For example, NLS sequences are described in Plank *et al.*, international PCT application, PCT/EP2000/011690, filed November 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference for its disclosure of exemplary nuclear localization sequences. In some embodiments, a NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 16) or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 17).

Nucleic acid molecule

[0227] The term "nucleic acid," as used herein, refers to a polymer of nucleotides. The polymer may include natural nucleosides (i.e., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine), nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C5 bromouridine, C5 fluorouridine, C5 iodouridine, C5 propynyl uridine, C5 propynyl cytidine, C5 methylcytidine, 7 deazaadenosine, 7 deazaguanosine, 8 oxoadenosine, 8 oxoguanosine, O(6) methylguanine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, dihydrouridine, methylpseudouridine, 1-methyl adenosine, 1-methyl guanosine, N6-methyl adenosine, and 2-thiocytidine), chemically modified bases, biologically modified bases (e.g., methylated bases), intercalated bases, modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, 2´-O-methylcytidine, arabinose, and hexose), or modified phosphate groups (e.g., phosphorothioates and 5′ N phosphoramidite linkages).

PEgRNA

[0228] As used herein, the terms "prime editing guide RNA" or "PEgRNA" or "extended guide RNA" refers to a specialized form of a guide RNA that has been modified to include one or more additional sequences for implementing the prime editing methods and compositions described herein. As described herein, the prime editing guide RNA comprise one or more "extended regions" of nucleic acid sequence. The extended regions may comprise, but are not limited to, single-stranded RNA or DNA. Further, the extended regions may occur at the 3´ end of a traditional guide RNA. In other arrangements, the extended regions may occur at the 5´ end of a traditional guide RNA. In still other arrangements, the extended region may occur at an intramolecular region of the traditional guide RNA, for example, in the gRNA core region which associates and/or binds to the napDNAbp. The extended region comprises a "DNA synthesis template" which encodes (by the polymerase of the prime editor) a single-stranded DNA which, in turn, has been designed to be (a) homologous with the endogenous target DNA to be edited, and (b) which comprises

at least one desired nucleotide change (e.g., a transition, a transversion, a deletion, or an insertion) to be introduced or integrated into the endogenous target DNA. The extended region may also comprise other functional sequence elements, such as, but not limited to, a "primer binding site" and a "spacer or linker" sequence, or other structural elements, such as, but not limited to aptamers, stem loops, hairpins, toe loops (e.g., a 3' toeloop), or an RNA-protein recruitment domain (e.g., MS2 hairpin). As used herein the "primer binding site" comprises a sequence that hybridizes to a single-strand DNA sequence having a 3′ end generated from the nicked DNA of the R-loop.

[0229] In certain embodiments, the PEgRNAs are represented by FIG. 3A, which shows a PEgRNA having a 5′ extension arm, a spacer, and a gRNA core. The 5′ extension further comprises in the 5′ to 3′ direction a reverse transcriptase template, a primer binding site, and a linker. As shown, the reverse transcriptase template may also be referred to more broadly as the "DNA synthesis template" where the polymerase of a prime editor described herein is not an RT, but another type of polymerase.

[0230] In certain other embodiments, the PEgRNAs are represented by FIG. 3B, which shows a PEgRNA having a 5′ extension arm, a spacer, and a gRNA core. The 5′ extension further comprises in the 5′ to 3′ direction a reverse transcriptase template, a primer binding site, and a linker. As shown, the reverse transcriptase template may also be referred to more broadly as the "DNA synthesis template" where the polymerase of a prime editor described herein is not an RT, but another type of polymerase.

[0231] In still other embodiments, the PEgRNAs are represented by FIG. 3D, which shows a PEgRNA having in the 5′ to 3′ direction a spacer (1), a gRNA core (2), and an extension arm (3). The extension arm (3) is at the 3′ end of the PEgRNA. The extension arm (3) further comprises in the 5′ to 3′ direction a "primer binding site" (A), an "edit template" (B), and a "homology arm" (C). The extension arm (3) may also comprise an optional modifier region at the 3′ and 5′ ends, which may be the same sequences or different sequences. In addition, the 3′ end of the PEgRNA may comprise a transcriptional terminator sequence. These sequence elements of the PEgRNAs are further described and defined herein.

[0232] In still other embodiments, the PEgRNAs are represented by FIG. 3E, which shows a PEgRNA having in the 5′ to 3′ direction an extension arm (3), a spacer (1), and a gRNA core (2). The extension arm (3) is at the 5′ end of the PEgRNA. The extension arm (3) further comprises in the 3′ to 5′ direction a "primer binding site" (A), an "edit template" (B), and a "homology arm" (C). The extension arm (3) may also comprise an optional modifier region

at the 3′ and 5′ ends, which may be the same sequences or different sequences. The PEgRNAs may also comprise a transcriptional terminator sequence at the 3′ end. These sequence elements of the PEgRNAs are further described and defined herein.

PE1

[0233] As used herein, "PE1" refers to a PE complex comprising a fusion protein comprising Cas9(H840A) and a wild type MMLV RT having the following structure: [NLS]-[Cas9(H840A)]-[linker]-[MMLV_RT(wt)] + a desired PEgRNA, wherein the PE fusion has the amino acid sequence of SEQ ID NO: 123, which is shown as follows;

MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN**
TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAK
VDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTD
KADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN
ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEI
TKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHA
ILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV
TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE
DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYA
HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELV
KVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV
ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNK
VLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGL
SELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLV
SDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYD
VRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW
DPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF
LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT
LIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESATPESSGGSSGGSS*TL*
NIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQY
PMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVED
IHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT
WTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALL
QTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF
LGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTK
PFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG
KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL
PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTET
EVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSE

*GKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTS TLLIENSSP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 123)

KEY:
NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 126)
33-AMINO ACID LINKER (SEQ ID NO: 127)
M-MLV reverse transcriptase (SEQ ID NO: 128).

PE2

[0234] As used herein, "PE2" refers to a PE complex comprising a fusion protein comprising Cas9(H840A) and a variant MMLV RT having the following structure: [NLS]-[Cas9(H840A)]-[linker]-[MMLV_RT(D200N)(T330P)(L603W)(T306K)(W313F)] + a desired PEgRNA, wherein the PE fusion has the amino acid sequence of SEQ ID NO: 134, which is shown as follows:

MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAK VDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTD KADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEI TKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHA ILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELV KVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNK VLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGL SELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLV SDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYD VRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW DPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESATPESSGGSSGGSS*TL NIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQY PMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVED IHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT WTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALL*

QTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF
LGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTK
PFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG
KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL
PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTET
EVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTS
EGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDT
*STLLIENSSP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 134)

KEY:
NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 137)
33-AMINO ACID LINKER (SEQ ID NO: 127)
M-MLV reverse transcriptase (SEQ ID NO: 139).

PE3

[0235] As used herein, "PE3" refers to PE2 plus a second-strand nicking guide RNA that complexes with the PE2 and introduces a nick in the non-edited DNA strand in order to induce preferential replacement of the edited strand.

PE3b

[0236] As used herein, "PE3b" refers to PE3 but wherein the second-strand nicking guide RNA is designed for temporal control such that the second strand nick is not introduced until after the installation of the desired edit. This is achieved by designing a gRNA with a spacer sequence that matches only the edited strand, but not the original allele. Using this strategy, referred to hereafter as PE3b, mismatches between the protospacer and the unedited allele should disfavor nicking by the sgRNA until after the editing event on the PAM strand takes place.

PE-short

[0237] As used herein, "PE-short" refers to a PE construct that is fused to a C-terminally truncated reverse transcriptase, and has the following amino acid sequence:

MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLG**
NTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMA
KVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST
DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPI
NASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF
DLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYID
GGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGE
LHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETI
TPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKV
KYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEIS

**GVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERL KTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFA NRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQIL KEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKD DSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTK AERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVI TLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY GDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIET NGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKL IARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSS FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELA LPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVIL ADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYT STKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESATPESS GGSSGGSS*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPL KATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQ DLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR DPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSEL DCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLT APALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRM VAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQF GPVVALNPATLLPLPEEGLQHNCLDNSRLIN*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 765)

KEY:
NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 157)
33-AMINO ACID LINKER 1 (SEQ ID NO: 127)
M-MLV TRUNCATED REVERSE TRANSCRIPTASE
(SEQ ID NO: 766)

Peptide tag

[0238] The term "peptide tag" refers to a peptide amino acid sequence that is genetically fused to a protein sequence to impart one or more functions onto the proteins that facilitate the manipulation of the protein for various purposes, such as, visualization, purification, solubilization, and separation, etc. Peptide tags can include various types of tags categorized by purpose or function, which may include "affinity tags" (to facilitate protein purification), "solubilization tags" (to assist in proper folding of proteins), "chromatography tags" (to alter chromatographic properties of proteins), "epitope tags" (to bind to high affinity antibodies), "fluorescence tags" (to facilitate visualization of proteins in a cell or in vitro).

Polymerase

[0239] As used herein, the term "polymerase" refers to an enzyme that synthesizes a nucleotide strand and which may be used in connection with the prime editor systems described herein. The polymerase can be a "template-dependent" polymerase (i.e., a polymerase which synthesizes a nucleotide strand based on the order of nucleotide bases of a template strand). The polymerase can also be a "template-independent" polymerase (i.e., a polymerase which synthesizes a nucleotide strand without the requirement of a template strand). A polymerase may also be further categorized as a "DNA polymerase" or an "RNA polymerase." In various embodiments, the prime editor system comprises a DNA polymerase. In various embodiments, the DNA polymerase can be a "DNA-dependent DNA polymerase" (i.e., whereby the template molecule is a strand of DNA). In such cases, the DNA template molecule can be a PEgRNA, wherein the extension arm comprises a strand of DNA. In such cases, the PEgRNA may be referred to as a chimeric or hybrid PEgRNA which comprises an RNA portion (i.e., the guide RNA components, including the spacer and the gRNA core) and a DNA portion (i.e., the extension arm). In various other embodiments, the DNA polymerase can be an "RNA-dependent DNA polymerase" (i.e., whereby the template molecule is a strand of RNA). In such cases, the PEgRNA is RNA, i.e., including an RNA extension. The term "polymerase" may also refer to an enzyme that catalyzes the polymerization of nucleotide (i.e., the polymerase activity). Generally, the enzyme will initiate synthesis at the 3'-end of a primer annealed to a polynucleotide template sequence (e.g., such as a primer sequence annealed to the primer binding site of a PEgRNA), and will proceed toward the 5' end of the template strand. A "DNA polymerase" catalyzes the polymerization of deoxynucleotides. As used herein in reference to a DNA polymerase, the term DNA polymerase includes a "functional fragment thereof". A "functional fragment thereof" refers to any portion of a wild-type or mutant DNA polymerase that encompasses less than the entire amino acid sequence of the polymerase and which retains the ability, under at least one set of conditions, to catalyze the polymerization of a polynucleotide. Such a functional fragment may exist as a separate entity, or it may be a constituent of a larger polypeptide, such as a fusion protein.

Prime editing

[0240] As used herein, the term "prime editing" refers to a novel approach for gene editing using napDNAbps, a polymerase (e.g., a reverse transcriptase), and specialized guide RNAs that include a DNA synthesis template for encoding desired new genetic information (or deleting genetic information) that is then incorporated into a target DNA sequence. Certain

embodiments of prime editing are described in the embodiments of FIGs. 1A-1H and FIG. 72(a)-72(c), among other figures.

[0241] Prime editing represents an entirely new platform for genome editing that is a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a nucleic acid programmable DNA binding protein ("napDNAbp") working in association with a polymerase (i.e., in the form of a fusion protein or otherwise provided *in trans* with the napDNAbp), wherein the prime editing system is programmed with a prime editing (PE) guide RNA ("PEgRNA") that both specifies the target site and templates the synthesis of the desired edit in the form of a replacement DNA strand by way of an extension (either DNA or RNA) engineered onto a guide RNA (e.g., at the 5′ or 3′ end, or at an internal portion of a guide RNA). The replacement strand containing the desired edit (e.g., a single nucleobase substitution) shares the same (or is homologous to) sequence as the endogenous strand (immediately downstream of the nick site) of the target site to be edited (with the exception that it includes the desired edit). Through DNA repair and/or replication machinery, the endogenous strand downstream of the nick site is replaced by the newly synthesized replacement strand containing the desired edit. In some cases, prime editing may be thought of as a "search-and-replace" genome editing technology since the prime editors, as described herein, not only search and locate the desired target site to be edited, but at the same time, encode a replacement strand containing a desired edit which is installed in place of the corresponding target site endogenous DNA strand. The prime editors of the present disclosure relate, in part, to the discovery that the mechanism of target-primed reverse transcription (TPRT) or "prime editing" can be leveraged or adapted for conducting precision CRISPR/Cas-based genome editing with high efficiency and genetic flexibility (e.g., as depicted in various embodiments of FIGs. 1A-1F). TPRT is naturally used by mobile DNA elements, such as mammalian non-LTR retrotransposons and bacterial Group II introns[28,29]. The inventors have herein used Cas protein-reverse transcriptase fusions or related systems to target a specific DNA sequence with a guide RNA, generate a single strand nick at the target site, and use the nicked DNA as a primer for reverse transcription of an engineered reverse transcriptase template that is integrated with the guide RNA. However, while the concept begins with prime editors that use reverse transcriptase as the DNA polymerase component, the prime editors described herein are not limited to reverse transcriptases but may include the use of virtually any DNA polymerase. Indeed, while the application throughout may refer to prime editors with "reverse transcriptases," it is set forth

here that reverse transcriptases are only one type of DNA polymerase that may work with prime editing. Thus, where ever the specification mentions a "reverse transcriptase," the person having ordinary skill in the art should appreciate that any suitable DNA polymerase may be used in place of the reverse transcriptase. Thus, in one aspect, the prime editors may comprise Cas9 (or an equivalent napDNAbp) which is programmed to target a DNA sequence by associating it with a specialized guide RNA (i.e., PEgRNA) containing a spacer sequence that anneals to a complementary protospacer in the target DNA. The specialized guide RNA also contains new genetic information in the form of an extension that encodes a replacement strand of DNA containing a desired genetic alteration which is used to replace a corresponding endogenous DNA strand at the target site. To transfer information from the PEgRNA to the target DNA, the mechanism of prime editing involves nicking the target site in one strand of the DNA to expose a 3′-hydroxyl group. The exposed 3′-hydroxyl group can then be used to prime the DNA polymerization of the edit-encoding extension on PEgRNA directly into the target site. In various embodiments, the extension—which provides the template for polymerization of the replacement strand containing the edit—can be formed from RNA or DNA. In the case of an RNA extension, the polymerase of the prime editor can be an RNA-dependent DNA polymerase (such as, a reverse transcriptase). In the case of a DNA extension, the polymerase of the prime editor may be a DNA-dependent DNA polymerase. The newly synthesized strand (i.e., the replacement DNA strand containing the desired edit) that is formed by the herein disclosed prime editors would be homologous to the genomic target sequence (i.e., have the same sequence as) except for the inclusion of a desired nucleotide change (e.g., a single nucleotide change, a deletion, or an insertion, or a combination thereof). The newly synthesized (or replacement) strand of DNA may also be referred to as a single strand DNA flap, which would compete for hybridization with the complementary homologous endogenous DNA strand, thereby displacing the corresponding endogenous strand. In certain embodiments, the system can be combined with the use of an error-prone reverse transcriptase enzyme (e.g., provided as a fusion protein with the Cas9 domain, or provided *in trans* to the Cas9 domain). The error-prone reverse transcriptase enzyme can introduce alterations during synthesis of the single strand DNA flap. Thus, in certain embodiments, error-prone reverse transcriptase can be utilized to introduce nucleotide changes to the target DNA. Depending on the error-prone reverse transcriptase that is used with the system, the changes can be random or non-random. Resolution of the hybridized intermediate (comprising the single strand DNA flap synthesized by the reverse transcriptase

hybridized to the endogenous DNA strand) can include removal of the resulting displaced flap of endogenous DNA (e.g., with a 5′ end DNA flap endonuclease, FEN1), ligation of the synthesized single strand DNA flap to the target DNA, and assimilation of the desired nucleotide change as a result of cellular DNA repair and/or replication processes. Because templated DNA synthesis offers single nucleotide precision for the modification of any nucleotide, including insertions and deletions, the scope of this approach is very broad and could foreseeably be used for myriad applications in basic science and therapeutics.

[0242] In various embodiments, prime editing operates by contacting a target DNA molecule (for which a change in the nucleotide sequence is desired to be introduced) with a nucleic acid programmable DNA binding protein (napDNAbp) complexed with a prime editing guide RNA (PEgRNA). In reference to FIG. 1G, the prime editing guide RNA (PEgRNA) comprises an extension at the 3′ or 5′ end of the guide RNA, or at an intramolecular location in the guide RNA and encodes the desired nucleotide change (e.g., single nucleotide change, insertion, or deletion). In step (a), the napDNAbp/extended gRNA complex contacts the DNA molecule and the extended gRNA guides the napDNAbp to bind to a target locus. In step (b), a nick in one of the strands of DNA of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand, i.e., the strand that is not hybridized to the guide RNA sequence, i.e., the "non-target strand." The nick, however, could be introduced in either of the strands. That is, the nick could be introduced into the R-loop "target strand" (i.e., the strand hybridized to the protospacer of the extended gRNA) or the "non-target strand" (i.e., the strand forming the single-stranded portion of the R-loop and which is complementary to the target strand). In step (c), the 3′ end of the DNA strand (formed by the nick) interacts with the extended portion of the guide RNA in order to prime reverse transcription (i.e., "target-primed RT"). In certain embodiments, the 3′ end DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA, i.e., the "reverse transcriptase priming sequence" or "primer binding site" on the PEgRNA. In step (d), a reverse transcriptase (or other suitable DNA polymerase) is introduced which synthesizes a single strand of DNA from the 3′ end of the primed site towards the 5′ end of the prime editing guide RNA. The DNA polymerase (e.g., reverse transcriptase) can be fused to the napDNAbp or alternatively can be provided *in trans* to the napDNAbp. This forms a single-strand DNA flap comprising the desired nucleotide change (e.g., the single base change,

insertion, or deletion, or a combination thereof) and which is otherwise homologous to the endogenous DNA at or adjacent to the nick site. In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap such that the desired nucleotide change becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap that forms once the 3′ single strand DNA flap invades and hybridizes to the endogenous DNA sequence. Without being bound by theory, the cells endogenous DNA repair and replication processes resolves the mismatched DNA to incorporate the nucleotide change(s) to form the desired altered product. The process can also be driven towards product formation with "second strand nicking," as exemplified in FIG. 1F. This process may introduce at least one or more of the following genetic changes: transversions, transitions, deletions, and insertions.

[0243] The term "prime editor (PE) system" or "prime editor (PE)" or "PE system" or "PE editing system" refers the compositions involved in the method of genome editing using target-primed reverse transcription (TPRT) describe herein, including, but not limited to the napDNAbps, reverse transcriptases, fusion proteins (e.g., comprising napDNAbps and reverse transcriptases), prime editing guide RNAs, and complexes comprising fusion proteins and prime editing guide RNAs, as well as accessory elements, such as second strand nicking components (e.g., second strand sgRNAs) and 5′ endogenous DNA flap removal endonucleases (e.g., FEN1) for helping to drive the prime editing process towards the edited product formation.

[0244] Although in the embodiments described thus far the PEgRNA constitutes a single molecule comprising a guide RNA (which itself comprises a spacer sequence and a gRNA core or scaffold) and a 5′ or 3′ extension arm comprising the primer binding site and a DNA synthesis template (e.g., see FIG. 3D, the PEgRNA may also take the form of two individual molecules comprised of a guide RNA and a *trans* prime editor RNA template (tPERT), which essentially houses the extension arm (including, in particular, the primer binding site and the DNA synthesis domain) and an RNA-protein recruitment domain (e.g., MS2 aptamer or hairpin) in the same molecule which becomes co-localized or recruited to a modified prime editor complex that comprises a tPERT recruiting protein (e.g., MS2cp protein, which binds to the MS2 aptamer). See FIG. 3G and FIG. 3H as an example of a tPERT that may be used with prime editing.

Prime editor

[0245] The term "prime editor" refers to the herein described fusion constructs comprising a napDNAbp (e.g., Cas9 nickase) and a reverse transcriptase and is capable of carrying out prime editing on a target nucleotide sequence in the presence of a PEgRNA (or "extended guide RNA"). The term "prime editor" may refer to the fusion protein or to the fusion protein complexed with a PEgRNA, and/or further complexed with a second-strand nicking sgRNA. In some embodiments, the prime editor may also refer to the complex comprising a fusion protein (reverse transcriptase fused to a napDNAbp), a PEgRNA, and a regular guide RNA capable of directing the second-site nicking step of the non-edited strand as described herein. In other embodiments, the reverse transcriptase component of the "primer editor" may be provided *in trans*.

Primer binding site

[0246] The term "primer binding site" or "the PBS" refers to the nucleotide sequence located on a PEgRNA as component of the extension arm (typically at the 3′ end of the extension arm) and serves to bind to the primer sequence that is formed after Cas9 nicking of the target sequence by the prime editor. As detailed elsewhere, when the Cas9 nickase component of a prime editor nicks one strand of the target DNA sequence, a 3′-ended ssDNA flap is formed, which serves a primer sequence that anneals to the primer binding site on the PEgRNA to prime reverse transcription. FIGs. 27 and 28 show embodiments of the primer binding site located on a 3′ and 5′ extension arm, respectively.

Promoter

[0247] The term "promoter" is art-recognized and refers to a nucleic acid molecule with a sequence recognized by the cellular transcription machinery and able to initiate transcription of a downstream gene. A promoter can be constitutively active, meaning that the promoter is always active in a given cellular context, or conditionally active, meaning that the promoter is only active in the presence of a specific condition. For example, a conditional promoter may only be active in the presence of a specific protein that connects a protein associated with a regulatory element in the promoter to the basic transcriptional machinery, or only in the absence of an inhibitory molecule. A subclass of conditionally active promoters are inducible promoters that require the presence of a small molecule "inducer" for activity. Examples of inducible promoters include, but are not limited to, arabinose-inducible promoters, Tet-on promoters, and tamoxifen-inducible promoters. A variety of constitutive, conditional, and inducible promoters are well known to the skilled artisan, and the skilled artisan will be able

to ascertain a variety of such promoters useful in carrying out the instant invention, which is not limited in this respect.

Protospacer

[0248] As used herein, the term "protospacer" refers to the sequence (~20 bp) in DNA adjacent to the PAM (protospacer adjacent motif) sequence. The protospacer shares the same sequence as the spacer sequence of the guide RNA. The guide RNA anneals to the complement of the protospacer sequence on the target DNA (specifically, one strand thereof, i.e., the "target strand" versus the "non-target strand" of the target DNA sequence). In order for Cas9 to function it also requires a specific protospacer adjacent motif (PAM) that varies depending on the bacterial species of the Cas9 gene. The most commonly used Cas9 nuclease, derived from *S. pyogenes*, recognizes a PAM sequence of NGG that is found directly downstream of the target sequence in the genomic DNA, on the non-target strand. The skilled person will appreciate that the literature in the state of the art sometimes refers to the "protospacer" as the ~20-nt target-specific guide sequence on the guide RNA itself, rather than referring to it as a "spacer." Thus, in some cases, the term "protospacer" as used herein may be used interchangeably with the term "spacer." The context of the description surrounding the appearance of either "protospacer" or "spacer" will help inform the reader as to whether the term is in reference to the gRNA or the DNA target.

Protospacer adjacent motif (PAM)

[0249] As used herein, the term "protospacer adjacent sequence" or "PAM" refers to an approximately 2-6 base pair DNA sequence that is an important targeting component of a Cas9 nuclease. Typically, the PAM sequence is on either strand, and is downstream in the 5′ to 3′ direction of Cas9 cut site. The canonical PAM sequence (i.e., the PAM sequence that is associated with the Cas9 nuclease of *Streptococcus pyogenes* or SpCas9) is 5′-NGG-3′ wherein "N" is any nucleobase followed by two guanine ("G") nucleobases. Different PAM sequences can be associated with different Cas9 nucleases or equivalent proteins from different organisms. In addition, any given Cas9 nuclease, e.g., SpCas9, may be modified to alter the PAM specificity of the nuclease such that the nuclease recognizes alternative PAM sequence.

[0250] For example, with reference to the canonical SpCas9 amino acid sequence is SEQ ID NO: 18, the PAM sequence can be modified by introducing one or more mutations, including (a) D1135V, R1335Q, and T1337R "the VQR variant", which alters the PAM specificity to NGAN or NGNG, (b) D1135E, R1335Q, and T1337R "the EQR variant", which alters the

PAM specificity to NGAG, and (c) D1135V, G1218R, R1335E, and T1337R "the VRER variant", which alters the PAM specificity to NGCG. In addition, the D1135E variant of canonical SpCas9 still recognizes NGG, but it is more selective compared to the wild type SpCas9 protein.

[0251] It will also be appreciated that Cas9 enzymes from different bacterial species (i.e., Cas9 orthologs) can have varying PAM specificities. For example, Cas9 from *Staphylococcus aureus* (SaCas9) recognizes NGRRT or NGRRN. In addition, Cas9 from *Neisseria meningitis* (NmCas) recognizes NNNNGATT. In another example, Cas9 from *Streptococcus thermophilis* (StCas9) recognizes NNAGAAW. In still another example, Cas9 from *Treponema denticola* (TdCas) recognizes NAAAAC. These are example are not meant to be limiting. It will be further appreciated that non-SpCas9s bind a variety of PAM sequences, which makes them useful when no suitable SpCas9 PAM sequence is present at the desired target cut site. Furthermore, non-SpCas9s may have other characteristics that make them more useful than SpCas9. For example, Cas9 from *Staphylococcus aureus* (SaCas9) is about 1 kilobase smaller than SpCas9, so it can be packaged into adeno-associated virus (AAV). Further reference may be made to Shah et al., "Protospacer recognition motifs: mixed identities and functional diversity," *RNA Biology*, 10(5): 891-899 (which is incorporated herein by reference).

Recombinase

[0252] The term "recombinase," as used herein, refers to a site-specific enzyme that mediates the recombination of DNA between recombinase recognition sequences, which results in the excision, integration, inversion, or exchange (*e.g.*, translocation) of DNA fragments between the recombinase recognition sequences. Recombinases can be classified into two distinct families: serine recombinases (*e.g.*, resolvases and invertases) and tyrosine recombinases (*e.g.*, integrases). Examples of serine recombinases include, without limitation, Hin, Gin, Tn3, β-six, CinH, ParA, γδ, Bxb1, ϕC31, TP901, TG1, φBT1, R4, φRV1, φFC1, MR11, A118, U153, and gp29. Examples of tyrosine recombinases include, without limitation, Cre, FLP, R, Lambda, HK101, HK022, and pSAM2. The serine and tyrosine recombinase names stem from the conserved nucleophilic amino acid residue that the recombinase uses to attack the DNA and which becomes covalently linked to the DNA during strand exchange. Recombinases have numerous applications, including the creation of gene knockouts/knock-ins and gene therapy applications. See, *e.g.*, Brown *et al.*, "Serine recombinases as tools for genome engineering." *Methods*. 2011;53(4):372-9; Hirano *et al.*, "Site-specific recombinases

as tools for heterologous gene integration." *Appl. Microbiol. Biotechnol.* 2011; 92(2):227-39; Chavez and Calos, "Therapeutic applications of the ΦC31 integrase system." *Curr. Gene Ther.* 2011;11(5):375-81; Turan and Bode, "Site-specific recombinases: from tag-and-target-to tag-and-exchange-based genomic modifications." *FASEB J.* 2011; 25(12):4088-107; Venken and Bellen, "Genome-wide manipulations of Drosophila melanogaster with transposons, Flp recombinase, and ΦC31 integrase." *Methods Mol. Biol.* 2012; 859:203-28; Murphy, "Phage recombinases and their applications." *Adv. Virus Res.* 2012; 83:367-414; Zhang *et al.*, "Conditional gene manipulation: Cre-ating a new biological era." *J. Zhejiang Univ. Sci. B.* 2012; 13(7):511-24; Karpenshif and Bernstein, "From yeast to mammals: recent advances in genetic control of homologous recombination." *DNA Repair (Amst).* 2012; 1;11(10):781-8; the entire contents of each are hereby incorporated by reference in their entirety. The recombinases provided herein are not meant to be exclusive examples of recombinases that can be used in embodiments of the invention. The methods and compositions of the invention can be expanded by mining databases for new orthogonal recombinases or designing synthetic recombinases with defined DNA specificities (See, *e.g.*, Groth *et al.*, "Phage integrases: biology and applications." *J. Mol. Biol.* 2004; 335, 667-678; Gordley *et al.*, "Synthesis of programmable integrases." *Proc. Natl. Acad. Sci. U S A.* 2009; 106, 5053-5058; the entire contents of each are hereby incorporated by reference in their entirety). Other examples of recombinases that are useful in the methods and compositions described herein are known to those of skill in the art, and any new recombinase that is discovered or generated is expected to be able to be used in the different embodiments of the invention. In some embodiments, the catalytic domains of a recombinase are fused to a nuclease-inactivated RNA-programmable nuclease (*e.g.*, dCas9, or a fragment thereof), such that the recombinase domain does not comprise a nucleic acid binding domain or is unable to bind to a target nucleic acid (*e.g.*, the recombinase domain is engineered such that it does not have specific DNA binding activity). Recombinases lacking DNA binding activity and methods for engineering such are known, and include those described by Klippel *et al.*, "Isolation and characterisation of unusual gin mutants." *EMBO J.* 1988; 7: 3983–3989: Burke *et al.*, "Activating mutations of Tn3 resolvase marking interfaces important in recombination catalysis and its regulation. *Mol Microbiol.* 2004; 51: 937–948; Olorunniji *et al.*, "Synapsis and catalysis by activated Tn3 resolvase mutants." *Nucleic Acids Res.* 2008; 36: 7181–7191; Rowland *et al.*, "Regulatory mutations in Sin recombinase support a structure-based model of the synaptosome." *Mol Microbiol.* 2009; 74: 282–298; Akopian *et*

al., "Chimeric recombinases with designed DNA sequence recognition." *Proc Natl Acad Sci USA.* 2003;100: 8688–8691; Gordley *et al.*, "Evolution of programmable zinc finger-recombinases with activity in human cells. *J Mol Biol.* 2007; 367: 802–813; Gordley *et al.*, "Synthesis of programmable integrases." *Proc Natl Acad Sci USA.* 2009;106: 5053–5058; Arnold *et al.*, "Mutants of Tn*3* resolvase which do not require accessory binding sites for recombination activity." *EMBO J.* 1999;18: 1407–1414; Gaj *et al.*, "Structure-guided reprogramming of serine recombinase DNA sequence specificity." *Proc Natl Acad Sci USA.* 2011;108(2):498-503; and Proudfoot *et al.*, "Zinc finger recombinases with adaptable DNA sequence specificity." *PLoS One.* 2011;6(4):e19537; the entire contents of each are hereby incorporated by reference. For example, serine recombinases of the resolvase-invertase group, *e.g.*, Tn3 and γδ resolvases and the Hin and Gin invertases, have modular structures with autonomous catalytic and DNA-binding domains (See, *e.g.*, Grindley *et al.*, "Mechanism of site-specific recombination." *Ann Rev Biochem.* 2006; 75: 567–605, the entire contents of which are incorporated by reference). The catalytic domains of these recombinases are thus amenable to being recombined with nuclease-inactivated RNA-programmable nucleases (*e.g.*, dCas9, or a fragment thereof) as described herein, *e.g.*, following the isolation of 'activated' recombinase mutants which do not require any accessory factors (*e.g.*, DNA binding activities) (See, *e.g.*, Klippel *et al.*, "Isolation and characterisation of unusual gin mutants." *EMBO J.* 1988; 7: 3983–3989: Burke *et al.*, "Activating mutations of Tn3 resolvase marking interfaces important in recombination catalysis and its regulation. *Mol Microbiol.* 2004; 51: 937–948; Olorunniji *et al.*, "Synapsis and catalysis by activated Tn3 resolvase mutants." *Nucleic Acids Res.* 2008; 36: 7181–7191; Rowland *et al.*, "Regulatory mutations in Sin recombinase support a structure-based model of the synaptosome." *Mol Microbiol.* 2009; 74: 282–298; Akopian *et al.*, "Chimeric recombinases with designed DNA sequence recognition." *Proc Natl Acad Sci USA.* 2003;100: 8688–8691). Additionally, many other natural serine recombinases having an N-terminal catalytic domain and a C-terminal DNA binding domain are known (*e.g.*, phiC31 integrase, TnpX transposase, IS607 transposase), and their catalytic domains can be co-opted to engineer programmable site-specific recombinases as described herein (See, *e.g.*, Smith *et al.*, "Diversity in the serine recombinases." *Mol Microbiol.* 2002;44: 299–307, the entire contents of which are incorporated by reference). Similarly, the core catalytic domains of tyrosine recombinases (*e.g.*, Cre, λ integrase) are known, and can be similarly co-opted to engineer programmable site-specific recombinases as described herein (See, *e.g.*, Guo *et al.*, "Structure of Cre

recombinase complexed with DNA in a site-specific recombination synapse." *Nature*. 1997; 389:40–46; Hartung *et al.*, "Cre mutants with altered DNA binding properties." *J Biol Chem* 1998; 273:22884–22891; Shaikh *et al.*, "Chimeras of the Flp and Cre recombinases: Tests of the mode of cleavage by Flp and Cre. *J Mol Biol*. 2000; 302:27–48; Rongrong *et al.*, "Effect of deletion mutation on the recombination activity of Cre recombinase." *Acta Biochim Pol*. 2005; 52:541–544; Kilbride *et al.*, "Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system." *J Mol Biol*. 2006; 355:185–195; Warren *et al.*, "A chimeric cre recombinase with regulated directionality." *Proc Natl Acad Sci USA*. 2008 105:18278–18283; Van Duyne, "Teaching Cre to follow directions." *Proc Natl Acad Sci USA*. 2009 Jan 6;106(1):4-5; Numrych *et al.*, "A comparison of the effects of single-base and triple-base changes in the integrase arm-type binding sites on the site-specific recombination of bacteriophage λ." *Nucleic Acids Res*. 1990; 18:3953–3959; Tirumalai *et al.*, "The recognition of core-type DNA sites by λ integrase." *J Mol Biol*. 1998; 279:513–527; Aihara *et al.*, "A conformational switch controls the DNA cleavage activity of λ integrase." *Mol Cell*. 2003; 12:187–198; Biswas *et al.*, "A structural basis for allosteric control of DNA recombination by λ integrase." *Nature*. 2005; 435:1059–1066; and Warren *et al.*, "Mutations in the amino-terminal domain of λ-integrase have differential effects on integrative and excisive recombination." *Mol Microbiol*. 2005; 55:1104–1112; the entire contents of each are incorporated by reference).

Recombinase recognition sequence

[0253] The term "recombinase recognition sequence", or equivalently as "RRS" or "recombinase target sequence", as used herein, refers to a nucleotide sequence target recognized by a recombinase and which undergoes strand exchange with another DNA molecule having a the RRS that results in excision, integration, inversion, or exchange of DNA fragments between the recombinase recognition sequences.

Recombine or recombination

[0254] The term "recombine," or "recombination," in the context of a nucleic acid modification (*e.g.*, a genomic modification), is used to refer to the process by which two or more nucleic acid molecules, or two or more regions of a single nucleic acid molecule, are modified by the action of a recombinase protein (*e.g.*, an inventive recombinase fusion protein provided herein). Recombination can result in, *inter alia*, the insertion, inversion, excision, or translocation of nucleic acids, *e.g.*, in or between one or more nucleic acid molecules. recombinase recognition sequences

Reverse transcriptase

[0255] The term "reverse transcriptase" describes a class of polymerases characterized as RNA-dependent DNA polymerases. All known reverse transcriptases require a primer to synthesize a DNA transcript from an RNA template. Historically, reverse transcriptase has been used primarily to transcribe mRNA into cDNA which can then be cloned into a vector for further manipulation. Avian myoblastosis virus (AMV) reverse transcriptase was the first widely used RNA-dependent DNA polymerase (Verma, Biochim. Biophys. Acta 473:1 (1977)). The enzyme has 5′-3′ RNA-directed DNA polymerase activity, 5′-3′ DNA-directed DNA polymerase activity, and RNase H activity. RNase H is a processive 5′ and 3′ ribonuclease specific for the RNA strand for RNA-DNA hybrids (Perbal, A Practical Guide to Molecular Cloning, New York: Wiley & Sons (1984)). Errors in transcription cannot be corrected by reverse transcriptase because known viral reverse transcriptases lack the 3′-5′ exonuclease activity necessary for proofreading (Saunders and Saunders, Microbial Genetics Applied to Biotechnology, London: Croom Helm (1987)). A detailed study of the activity of AMV reverse transcriptase and its associated RNase H activity has been presented by Berger et al., Biochemistry 22:2365-2372 (1983). Another reverse transcriptase which is used extensively in molecular biology is reverse transcriptase originating from Moloney murine leukemia virus (M-MLV). See, e.g., Gerard, G. R., DNA 5:271-279 (1986) and Kotewicz, M. L., et al., Gene 35:249-258 (1985). M-MLV reverse transcriptase substantially lacking in RNase H activity has also been described. See, e.g., U.S. Pat. No. 5,244,797. The invention contemplates the use of any such reverse transcriptases, or variants or mutants thereof.

[0256] In addition, the invention contemplates the use of reverse transcriptases which are error-prone, i.e., which may be referred to as error-prone reverse transcriptases or reverse transcriptases which do not support high fidelity incorporation of nucleotides during polymerization. During synthesis of the single-strand DNA flap based on the RT template integrated with the guide RNA, the error-prone reverse transcriptase can introduce one or more nucleotides which are mismatched with the RT template sequence, thereby introducing changes to the nucleotide sequence through erroneous polymerization of the single-strand DNA flap. These errors introduced during synthesis of the single strand DNA flap then become integrated into the double strand molecule through hybridization to the corresponding endogenous target strand, removal of the endogenous displaced strand, ligation, and then through one more round of endogenous DNA repair and/or sequencing processes.

Reverse transcription

[0257] As used herein, the term "reverse transcription" indicates the capability of enzyme to synthesize DNA strand (that is, complementary DNA or cDNA) using RNA as a template. In some embodiments, the reverse transcription can be "error-prone reverse transcription," which refers to the properties of certain reverse transcriptase enzymes which are error-prone in their DNA polymerization activity.

PACE

[0258] The term "phage-assisted continuous evolution (PACE)," as used herein, refers to continuous evolution that employs phage as viral vectors. The general concept of PACE technology has been described, for example, in International PCT Application, PCT/US2009/056194, filed September 8, 2009, published as WO 2010/028347 on March 11, 2010; International PCT Application, PCT/US2011/066747, filed December 22, 2011, published as WO 2012/088381 on June 28, 2012; U.S. Application, U.S. Patent No. 9,023,594, issued May 5, 2015, International PCT Application, PCT/US2015/012022, filed January 20, 2015, published as WO 2015/134121 on September 11, 2015, and International PCT Application, PCT/US2016/027795, filed April 15, 2016, published as WO 2016/168631 on October 20, 2016, the entire contents of each of which are incorporated herein by reference.

Phage

[0259] The term "phage," as used herein interchangeably with the term "bacteriophage," refers to a virus that infects bacterial cells. Typically, phages consist of an outer protein capsid enclosing genetic material. The genetic material can be ssRNA, dsRNA, ssDNA, or dsDNA, in either linear or circular form. Phages and phage vectors are well known to those of skill in the art and non-limiting examples of phages that are useful for carrying out the PACE methods provided herein are λ (Lysogen), T2, T4, T7, T12, R17, M13, MS2, G4, P1, P2, P4, Phi X174, N4, Φ6, and Φ29. In certain embodiments, the phage utilized in the present invention is M13. Additional suitable phages and host cells will be apparent to those of skill in the art and the invention is not limited in this aspect. For an exemplary description of additional suitable phages and host cells, see Elizabeth Kutter and Alexander Sulakvelidze: Bacteriophages: Biology and Applications. CRC Press; 1st edition (December 2004), ISBN: 0849313368; Martha R. J. Clokie and Andrew M. Kropinski: Bacteriophages: Methods and Protocols, Volume 1: Isolation, Characterization, and Interactions (Methods in Molecular Biology) Humana Press; 1st edition (December, 2008), ISBN: 1588296822; Martha R. J.

Clokie and Andrew M. Kropinski: Bacteriophages: Methods and Protocols, Volume 2: Molecular and Applied Aspects (Methods in Molecular Biology) Humana Press; 1st edition (December 2008), ISBN: 1603275649; all of which are incorporated herein in their entirety by reference for disclosure of suitable phages and host cells as well as methods and protocols for isolation, culture, and manipulation of such phages).

Protein, peptide, and polypeptide

[0260] The terms "protein," "peptide," and "polypeptide" are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide may refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. A protein, peptide, or polypeptide may also be a single molecule or may be a multi-molecular complex. A protein, peptide, or polypeptide may be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide may be naturally occurring, recombinant, or synthetic, or any combination thereof. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

Protein splicing

[0261] The term "protein splicing," as used herein, refers to a process in which a sequence, an intein (or split inteins, as the case may be), is excised from within an amino acid sequence, and the remaining fragments of the amino acid sequence, the exteins, are ligated via an amide bond to form a continuous amino acid sequence. The term "trans" protein splicing refers to the specific case where the inteins are split inteins and they are located on different proteins.

Second-strand nicking

[0262] The resolution of heteroduplex DNA (i.e., containing one edited and one non-edited strand) formed as a result of prime editing determines long-term editing outcomes. In words, a goal of prime editing is to resolve the heteroduplex DNA (the edited strand paired with the endogenous non-edited strand) formed as an intermediate of PE by permanently integrating the edited strand into the complement, endogenous strand. The approach of "second-strand nicking" can be used herein to help drive the resolution of heteroduplex DNA in favor of permanent integration of the edited strand into the DNA molecule. As used herein, the concept of "second-strand nicking" refers to the introduction of a second nick at a location downstream of the first nick (i.e., the initial nick site that provides the free 3′ end for use in priming of the reverse transcriptase on the extended portion of the guide RNA), preferably on the unedited strand. In certain embodiments, the first nick and the second nick are on opposite strands. In other embodiments, the first nick and the second nick are on opposite strands. In yet another embodiment, the first nick is on the non-target strand (i.e., the strand that forms the single strand portion of the R-loop), and the second nick is on the target strand. In still other embodiments, the first nick is on the edited strand, and the second nick is on the unedited strand. The second nick can be positioned at least 5 nucleotides downstream of the first nick, or at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 or more nucleotides downstream of the first nick. The second nick, in certain embodiments, can be introduced between about 5-150 nucleotides on the unedited strand away from the site of the PEgRNA-induced nick, or between about 5-140, or between about 5-130, or between about 5-120, or between about 5-110, or between about 5-100, or between about 5-90, or between about 5-80, or between about 5-70, or between about 5-60, or between about 5-50, or between about 5-40, or between about 5-30, or between about 5-20, or between about 5-10. In one embodiment, the second nick is introduced between 14-116 nucleotides away from the PEgRNA-induced nick. Without being bound by theory, the second nick induces the cell's endogenous DNA repair and replication processes towards replacement or editing of the unedited strand, thereby permanently installing the edited sequence on both strands and resolving the heteroduplex that is formed as a result of PE. In some embodiments, the edited strand is the non-target strand and the unedited strand is the target strand. In other embodiments, the edited strand is the target strand, and the unedited strand is the non-target strand.

Sense strand

[0263] In genetics, a "sense" strand is the segment within double-stranded DNA that runs from 5' to 3', and which is complementary to the antisense strand of DNA, or template strand, which runs from 3' to 5'. In the case of a DNA segment that encodes a protein, the sense strand is the strand of DNA that has the same sequence as the mRNA, which takes the antisense strand as its template during transcription, and eventually undergoes (typically, not always) translation into a protein. The antisense strand is thus responsible for the RNA that is later translated to protein, while the sense strand possesses a nearly identical makeup to that of the mRNA. Note that for each segment of dsDNA, there will possibly be two sets of sense and antisense, depending on which direction one reads (since sense and antisense is relative to perspective). It is ultimately the gene product, or mRNA, that dictates which strand of one segment of dsDNA is referred to as sense or antisense.

[0264] In the context of a PEgRNA, the first step is the synthesis of a single-strand complementary DNA (i.e., the 3′ ssDNA flap, which becomes incorporated) oriented in the 5′ to 3′ direction which is templated off of the PEgRNA extension arm. Whether the 3′ ssDNA flap should be regarded as a sense or antisense strand depends on the direction of transcription since it well accepted that both strands of DNA may serve as a template for transcription (but not at the same time). Thus, in some embodiments, the 3′ ssDNA flap (which overall runs in the 5′ to 3′ direction) will serve as the sense strand because it is the coding strand. In other embodiments, the 3′ ssDNA flap (which overall runs in the 5′ to 3′ direction) will serve as the antisense strand and thus, the template for transcription.

Spacer sequence

[0265] As used herein, the term "spacer sequence" in connection with a guide RNA or a PEgRNA refers to the portion of the guide RNA or PEgRNA of about 20 nucleotides which contains a nucleotide sequence that is complementary to the protospacer sequence in the target DNA sequence. The spacer sequence anneals to the protospacer sequence to form a ssRNA/ssDNA hybrid structure at the target site and a corresponding R loop ssDNA structure of the endogenous DNA strand that is complementary to the protospacer sequence.

Subject

[0266] The term "subject," as used herein, refers to an individual organism, for example, an individual mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a rodent. In some embodiments, the subject is a sheep, a goat, a cattle, a cat, or a dog. In some embodiments, the subject is a vertebrate, an

amphibian, a reptile, a fish, an insect, a fly, or a nematode. In some embodiments, the subject is a research animal. In some embodiments, the subject is genetically engineered, e.g., a genetically engineered non-human subject. The subject may be of either sex and at any stage of development.

Split intein

[0267] Although inteins are most frequently found as a contiguous domain, some exist in a naturally split form. In this case, the two fragments are expressed as separate polypeptides and must associate before splicing takes place, so-called protein trans-splicing.

[0268] An exemplary split intein is the *Ssp* DnaE intein, which comprises two subunits, namely, DnaE-N and DnaE-C. The two different subunits are encoded by separate genes, namely *dnaE-n* and *dnaE-c*, which encode the DnaE-N and DnaE-C subunits, respectively. DnaE is a naturally occurring split intein in *Synechocytis sp.* PCC6803 and is capable of directing trans-splicing of two separate proteins, each comprising a fusion with either DnaE-N or DnaE-C.

[0269] Additional naturally occurring or engineered split-intein sequences are known in the or can be made from whole-intein sequences described herein or those available in the art. Examples of split-intein sequences can be found in Stevens et al., "A promiscuous split intein with expanded protein engineering applications," PNAS, 2017, Vol.114: 8538-8543; Iwai et al., "Highly efficient protein trans-splicing by a naturally split DnaE intein from Nostc punctiforme, FEBS Lett, 580: 1853-1858, each of which are incorporated herein by reference. Additional split intein sequences can be found, for example, in WO 2013/045632, WO 2014/055782, WO 2016/069774, and EP2877490, the contents each of which are incorporated herein by reference.

[0270] In addition, protein splicing in trans has been described *in vivo* and *in vitro* (Shingledecker, et al., *Gene* 207:187 (1998), Southworth, et al., *EMBO J.* 17:918 (1998); Mills, et al., *Proc. Natl. Acad. Sci. USA,* 95:3543-3548 (1998); Lew, et al., *J. Biol. Chem.,* 273:15887-15890 (1998); Wu, et al., *Biochim. Biophys. Acta* 35732:1 (1998b), Yamazaki, et al., *J. Am. Chem. Soc.* 120:5591 (1998), Evans, et al., *J. Biol. Chem.* 275:9091 (2000); Otomo, et al., *Biochemistry* 38:16040-16044 (1999); Otomo, et al., *J. Biolmol. NMR* 14:105-114 (1999); Scott, et al., *Proc. Natl. Acad. Sci. USA* 96:13638-13643 (1999)) and provides the opportunity to express a protein as to two inactive fragments that subsequently undergo ligation to form a functional product, e.g., as shown in FIGs. 66 and 67 with regard to the formation of a complete PE fusion protein from two separately-expressed halves.

Target site

[0271] The term "target site" refers to a sequence within a nucleic acid molecule that is edited by a prime editor (PE) disclosed herein. The target site further refers to the sequence within a nucleic acid molecule to which a complex of the prime editor (PE) and gRNA binds.

tPERT

[0272] See definition for "*trans* prime editor RNA template (tPERT)."

Temporal second-strand nicking

[0273] As used herein, the term "temporal second-strand nicking" refers to a variant of second strand nicking whereby the installation of the second nick in the unedited strand occurs only after the desired edit is installed in the edited strand. This avoids concurrent nicks on both strands that could lead to double-stranded DNA breaks. The second-strand nicking guide RNA is designed for temporal control such that the second strand nick is not introduced until after the installation of the desired edit. This is achieved by designing a gRNA with a spacer sequence that matches only the edited strand, but not the original allele. Using this strategy, mismatches between the protospacer and the unedited allele should disfavor nicking by the sgRNA until after the editing event on the PAM strand takes place.

***Trans* prime editing**

[0274] As used herein, the term "*trans* prime editing" refers to a modified form of prime editing that utilizes a split PEgRNA, i.e., wherein the PEgRNA is separated into two separate molecules: an sgRNA and a *trans* prime editing RNA template (tPERT). The sgRNA serves to target the prime editor (or more generally, to target the napDNAbp component of the prime editor) to the desired genomic target site, while the tPERT is used by the polymerase (e.g., a reverse transcriptase) to write new DNA sequence into the target locus once the tPERT is recruited *in trans* to the prime editor by the interaction of binding domains located on the prime editor and on the tPERT. In one embodiment, the binding domains can include RNA-protein recruitment moieties, such as a MS2 aptamer located on the tPERT and an MS2cp protein fused to the prime editor. An advantage of *trans* prime editing is that by separating the DNA synthesis template from the guide RNA, one can potentially use longer length templates.

[0275] An embodiment of *trans* prime editing is shown in FIGs. 3G and 3H. FIG. 3G shows the composition of the *trans* prime editor complex on the left ("RP-PE:gRNA complex), which comprises an napDNAbp fused to each of a polymerase (e.g., a reverse transcriptase) and a rPERT recruiting protein (e.g., MS2sc), and which is complexed with a guide RNA.

FIG. 3G further shows a separate tPERT molecule, which comprises the extension arm features of a PEgRNA, including the DNA synthesis template and the primer binding sequence. The tPERT molecule also includes an RNA-protein recruitment domain (which, in this case, is a stem loop structure and can be, for example, MS2 aptamer). As depicted in the process described in FIG. 3H, the RP-PE:gRNA complex binds to and nicks the target DNA sequence. Then, the recruiting protein (RP) recruits a tPERT to co-localize to the prime editor complex bound to the DNA target site, thereby allowing the primer binding site to bind to the primer sequence on the nicked strand, and subsequently, allowing the polymerase (e.g., RT) to synthesize a single strand of DNA against the DNA synthesis template up through the 5′ of the tPERT.

[0276] While the tPERT is shown in FIG. 3G and FIG. 3H as comprising the PBS and DNA synthesis template on the 5′ end of the RNA-protein recruitment domain, the tPERT in other configurations may be designed with the PBS and DNA synthesis template located on the 3′ end of the RNA-protein recruitment domain. However, the tPERT with the 5' extension has the advantage that synthesis of the single strand of DNA will naturally terminate at the 5' end of the tPERT and thus, does not risk using any portion of the RNA-protein recruitment domain as a template during the DNA synthesis stage of prime editing.

***Trans* prime editor RNA template (tPERT)**

[0277] As used herein, a "trans prime editor RNA template (tPERT)" refers to a component used in trans prime editing, a modified version of prime editing which operates by separating the PEgRNA into two distinct molecules: a guide RNA and a tPERT molecule. The tPERT molecule is programmed to co-localize with the prime editor complex at a target DNA site, bringing the primer binding site and the DNA synthesis template to the prime editor in trans. For example, see FIG. 3G for an embodiment of a trans prime editor (tPE) which shows a two-component system comprising (1) an RP-PE:gRNA complex and (2) a tPERT that includes the primer binding site and the DNA synthesis template joined to an RNA-protein recruitment domain, wherein the RP (recruiting protein) component of the RP-PE:gRNA complex recruits the tPERT to a target site to be edited, thereby associating the PBS and DNA synthesis template with the prime editor in trans. Said another way, the tPERT is engineered to contain (all or part of) the extension arm of a PEgRNA, which includes the primer binding site and the DNA synthesis template.

Transitions

[0278] As used herein, "transitions" refer to the interchange of purine nucleobases (A ↔ G) or the interchange of pyrimidine nucleobases (C ↔ T). This class of interchanges involves nucleobases of similar shape. The compositions and methods disclosed herein are capable of inducing one or more transitions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule. These changes involve A ↔ G, G ↔ A, C ↔ T, or T ↔ C. In the context of a double-strand DNA with Watson-Crick paired nucleobases, transversions refer to the following base pair exchanges: A:T ↔ G:C, G:G ↔ A:T, C:G ↔ T:A, or T:A↔ C:G. The compositions and methods disclosed herein are capable of inducing one or more transitions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule, as well as other nucleotide changes, including deletions and insertions.

Transversions

[0279] As used herein, "transversions" refer to the interchange of purine nucleobases for pyrimidine nucleobases, or in the reverse and thus, involve the interchange of nucleobases with dissimilar shape. These changes involve T ↔ A, T↔ G, C ↔ G, C ↔ A, A ↔ T, A ↔ C, G ↔ C, and G ↔ T. In the context of a double-strand DNA with Watson-Crick paired nucleobases, transversions refer to the following base pair exchanges: T:A ↔ A:T, T:A ↔ G:C, C:G ↔ G:C, C:G ↔ A:T, A:T ↔ T:A, A:T ↔ C:G, G:C ↔ C:G, and G:C ↔ T:A. The compositions and methods disclosed herein are capable of inducing one or more transversions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule, as well as other nucleotide changes, including deletions and insertions.

Treatment

[0280] The terms "treatment," "treat," and "treating," refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. As used herein, the terms "treatment," "treat," and "treating" refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed and/or after a disease has been diagnosed. In other embodiments, treatment may be administered in the absence of symptoms, *e.g.*, to prevent or delay onset of a symptom or inhibit onset or progression of a disease. For example, treatment may be

administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example, to prevent or delay their recurrence.

Trinucleotide repeat disorder

[0281] As used herein, a "trinucleotide repeat disorder" (or alternatively, "expansion repeat disorder" or "repeat expansion disorder") refers to a set of genetic disorders which are cause by "trinucleotide repeat expansion," which is a kind of mutation where a certain trinucleotide repeats in certain genes or introns. Trinucleotide repeats were once thought to be commonplace iterations in the genome, but the 1990s clarified these disorders. These apparently 'benign' stretches of DNA can sometimes expand and cause disease. Several defining features are shared amongst disorders caused by trinucleotide repeat expansions. First, the mutant repeats show both somatic and germline instability and, more frequently, they expand rather than contract in successive transmissions. Secondly, an earlier age of onset and increasing severity of phenotype in subsequent generations (anticipation) generally are correlated with larger repeat length. Finally, the parental origin of the disease allele can often influence anticipation, with paternal transmissions carrying a greater risk of expansion for many of these disorders.

[0282] Triplet expansion is thought to be caused by slippage during DNA replication. Due to the repetitive nature of the DNA sequence in these regions 'loop out' structures may form during DNA replication while maintaining complementary base pairing between the parent strand and daughter strand being synthesized. If the loop out structure is formed from sequence on the daughter strand this will result in an increase in the number of repeats. However, if the loop out structure is formed on the parent strand a decrease in the number of repeats occurs. It appears that expansion of these repeats is more common than reduction. Generally the larger the expansion the more likely they are to cause disease or increase the severity of disease. This property results in the characteristic of anticipation seen in trinucleotide repeat disorders. Anticipation describes the tendency of age of onset to decrease and severity of symptoms to increase through successive generations of an affected family due to the expansion of these repeats.

[0283] Nucleotide repeat disorders may include those in which the triplet repeat occurs in a non-coding region (i.e., a non-coding trinucleotide repeat disorder) or in a coding region

[0284] The prime editor (PE) system described herein may use to treat nucleotide repeat disorders, which may include fragile X syndrome (FRAXA), fragile XE MR (FRAXE), Freidreich ataxia (FRDA), myotonic dystrophy (DM), spinocerebellar ataxia type 8 (SCA8), and spinocerebellar ataxia type 12 (SCA12), among others.

Upstream

[0285] As used herein, the terms "upstream" and "downstream" are terms of relativity that define the linear position of at least two elements located in a nucleic acid molecule (whether single or double-stranded) that is orientated in a 5′-to-3′ direction. In particular, a first element is upstream of a second element in a nucleic acid molecule where the first element is positioned somewhere that is 5' to the second element. For example, a SNP is upstream of a Cas9-induced nick site if the SNP is on the 5' side of the nick site. Conversely, a first element is downstream of a second element in a nucleic acid molecule where the first element is positioned somewhere that is 3' to the second element. For example, a SNP is downstream of a Cas9-induced nick site if the SNP is on the 3' side of the nick site. The nucleic acid molecule can be a DNA (double or single stranded). RNA (double or single stranded), or a hybrid of DNA and RNA. The analysis is the same for single strand nucleic acid molecule and a double strand molecule since the terms upstream and downstream are in reference to only a single strand of a nucleic acid molecule, except that one needs to select which strand of the double stranded molecule is being considered. Often, the strand of a double stranded DNA which can be used to determine the positional relativity of at least two elements is the "sense" or "coding" strand. In genetics, a "sense" strand is the segment within double-stranded DNA that runs from 5' to 3', and which is complementary to the antisense strand of DNA, or template strand, which runs from 3' to 5'. Thus, as an example, a SNP nucleobase is "downstream" of a promoter sequence in a genomic DNA (which is double-stranded) if the SNP nucleobase is on the 3' side of the promoter on the sense or coding strand.

Variant

[0286] As used herein the term "variant" should be taken to mean the exhibition of qualities that have a pattern that deviates from what occurs in nature, e.g., a variant Cas9 is a Cas9 comprising one or more changes in amino acid residues as compared to a wild type Cas9 amino acid sequence. The term "variant" encompasses homologous proteins having at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99% percent identity with a reference sequence and having the same or substantially the same functional activity or activities as the reference sequence. The term also encompasses mutants,

truncations, or domains of a reference sequence, and which display the same or substantially the same functional activity or activities as the reference sequence.

Vector

[0287] The term "vector," as used herein, refers to a nucleic acid that can be modified to encode a gene of interest and that is able to enter into a host cell, mutate and replicate within the host cell, and then transfer a replicated form of the vector into another host cell. Exemplary suitable vectors include viral vectors, such as retroviral vectors or bacteriophages and filamentous phage, and conjugative plasmids. Additional suitable vectors will be apparent to those of skill in the art based on the instant disclosure.

Wild type

[0288] As used herein the term "wild type" is a term of the art understood by skilled persons and means the typical form of an organism, strain, gene or characteristic as it occurs in nature as distinguished from mutant or variant forms.

5′ endogenous DNA flap

[0289] As used herein, the term "5′ endogenous DNA flap" refers to the strand of DNA situated immediately downstream of the PE-induced nick site in the target DNA. The nicking of the target DNA strand by PE exposes a 3′ hydroxyl group on the upstream side of the nick site and a 5′ hydroxyl group on the downstream side of the nick site. The endogenous strand ending in the 3′ hydroxyl group is used to prime the DNA polymerase of the prime editor (e.g., wherein the DNA polymerase is a reverse transcriptase). The endogenous strand on the downstream side of the nick site and which begins with the exposed 5′ hydroxyl group is referred to as the "5′ endogenous DNA flap" and is ultimately removed and replaced by the newly synthesized replacement strand (i.e., "3′ replacement DNA flap") the encoded by the extension of the PEgRNA.

5′ endogenous DNA flap removal

[0290] As used herein, the term "5′ endogenous DNA flap removal" or "5′ flap removal" refers to the removal of the 5′ endogenous DNA flap that forms when the RT-synthesized single-strand DNA flap competitively invades and hybridizes to the endogenous DNA, displacing the endogenous strand in the process. Removing this endogenous displaced strand can drive the reaction towards the formation of the desired product comprising the desired nucleotide change. The cell's own DNA repair enzymes may catalyze the removal or excision of the 5′ endogenous flap (e.g., a flap endonuclease, such as EXO1 or FEN1). Also, host cells may be transformed to express one or more enzymes that catalyze the removal of

said 5′ endogenous flaps, thereby driving the process toward product formation (e.g., a flap endonuclease). Flap endonucleases are known in the art and can be found described in Patel et al., "Flap endonucleases pass 5′-flaps through a flexible arch using a disorder-thread-order mechanism to confer specificity for free 5′-ends," *Nucleic Acids Research*, 2012, 40(10): 4507-4519 and Tsutakawa et al., "Human flap endonuclease structures, DNA double-base flipping, and a unified understanding of the FEN1 superfamily," *Cell*, 2011, 145(2): 198-211 (each of which are incorporated herein by reference).

3′ replacement DNA flap

[0291] As used herein, the term "3′ replacement DNA flap" or simply, "replacement DNA flap," refers to the strand of DNA that is synthesized by the prime editor and which is encoded by the extension arm of the prime editor PEgRNA. More in particular, the 3′ replacement DNA flap is encoded by the polymerase template of the PEgRNA. The 3′ replacement DNA flap comprises the same sequence as the 5′ endogenous DNA flap except that it also contains the edited sequence (e.g., single nucleotide change). The 3′ replacement DNA flap anneals to the target DNA, displacing or replacing the 5′ endogenous DNA flap (which can be excised, for example, by a 5′ flap endonuclease, such as FEN1 or EXO1) and then is ligated to join the 3′ end of the 3′ replacement DNA flap to the exposed 5′ hydoxyl end of endogenous DNA (exposed after excision of the 5′ endogenous DNA flap, thereby reforming a phosophodiester bond and installing the 3′ replacement DNA flap to form a heteroduplex DNA containing one edited strand and one unedited strand. DNA repair processes resolve the heteroduplex by copying the information in the edited strand to the complementary strand permanently installs the edit in to the DNA. This resolution process can be driven further to completion by nicking the unedited strand, i.e., by way of "second-strand nicking," as described herein.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0292] Adoption of the clustered regularly interspaced short palindromic repeat (CRISPR) system for genome editing has revolutionized the life sciences[1–3]. Although gene disruption using CRISPR is now routine, the precise installation of single nucleotide edits remains a major challenge, despite being necessary for studying or correcting a large number of disease-causative mutations. Homology directed repair (HDR) is capable of achieving such edits, but suffers from low efficiency (often <5%), a requirement for donor DNA repair templates, and deleterious effects of double-stranded DNA break (DSB) formation. Recently, Prof. David Liu *et al.*'s laboratory developed base editing, which achieves efficient single

nucleotide editing without DSBs. Base editors (BEs) combine the CRISPR system with base-modifying deaminase enzymes to convert target C•G or A•T base pairs to A•T or G•C, respectively[4–6]. Although already widely used by researchers worldwide, current BEs enable only four of the twelve possible base pair conversions and are unable to correct small insertions or deletions. Moreover, the targeting scope of base editing is limited by the editing of non-target C or A bases adjacent to the target base ("bystander editing") and by the requirement that a PAM sequence exist 15±2 bp from the target base. Overcoming these limitations would therefore greatly broaden the basic research and therapeutic applications of genome editing.

[0293] The present disclosure proposes a new precision editing approach that offers many of the benefits of base editing—namely, avoidance of double strand breaks and donor DNA repair templates—while overcoming its major limitations. The proposed approach described herein achieves the direct installation of edited DNA strands at target genomic sites using target-primed reverse transcription (TPRT). In the design discussed herein, CRISPR guide RNA (gRNA) will be engineered to carry a reverse transcriptase (RT) template sequence encoding a single-stranded DNA comprising a desired nucleotide change. The CRISPR nuclease (Cas9)-nicked target site DNA will serve as the primer for reverse transcription of the template sequence on the modified gRNA, allowing for direct incorporation of any desired nucleotide edit.

[0294] Accordingly, the present invention relates in part to the discovery that the mechanism of target-primed reverse transcription (TPRT) can be leveraged or adapted for conducting precision CRISPR/Cas-based genome editing with high efficiency and genetic flexibility (e.g., as depicted in various embodiments of FIGs. 1A-1F). The inventors have proposed herein to use Cas protein-reverse transcriptase fusions to target a specific DNA sequence with a modified guide RNA ("an extended guide RNA"), generate a single strand nick at the target site, and use the nicked DNA as a primer for reverse transcription of an engineered reverse transcriptase template that is integrated into the extended guide RNA. The newly synthesized strand would be homologous to the genomic target sequence except for the inclusion of a desired nucleotide change (e.g., a single nucleotide change, a deletion, or an insertion, or a combination thereof). The newly synthesize strand of DNA may be referred to as a single strand DNA flap, which would compete for hybridization with the complementary homologous endogenous DNA strand, thereby displacing the corresponding endogenous strand. Resolution of this hybridized intermediate can include removal of the resulting

displaced flap of endogenous DNA (e.g., with a 5′ end DNA flap endonuclease, FEN1), ligation of the synthesized single strand DNA flap to the target DNA, and assimilation of the desired nucleotide change as a result of cellular DNA repair and/or replication processes. Because templated DNA synthesis offers single nucleotide precision, the scope of this approach is very broad and could foreseeably be used for myriad applications in basic science and therapeutics.

[1] napDNAbp

[0295] The prime editors and *trans* prime editors described herein may comprise a nucleic acid programmable DNA binding protein (napDNAbp).

[0296] In one aspect, a napDNAbp can be associated with or complexed with at least one guide nucleic acid (e.g., guide RNA or a PEgRNA), which localizes the napDNAbp to a DNA sequence that comprises a DNA strand (i.e., a target strand) that is complementary to the guide nucleic acid, or a portion thereof (e.g., the spacer of a guide RNA which anneals to the protospacer of the DNA target). In other words, the guide nucleic-acid "programs" the napDNAbp (e.g., Cas9 or equivalent) to localize and bind to complementary sequence of the protospacer in the DNA.

[0297] Any suitable napDNAbp may be used in the prime editors described herein. In various embodiments, the napDNAbp may be any Class 2 CRISPR-Cas system, including any type II, type V, or type VI CRISPR-Cas enzyme. Given the rapid development of CRISPR-Cas as a tool for genome editing, there have been constant developments in the nomenclature used to describe and/or identify CRISPR-Cas enzymes, such as Cas9 and Cas9 orthologs. This application references CRISPR-Cas enzymes with nomenclature that may be old and/or new. The skilled person will be able to identify the specific CRISPR-Cas enzyme being referenced in this Application based on the nomenclature that is used, whether it is old (i.e., "legacy") or new nomenclature. CRISPR-Cas nomenclature is extensively discussed in Makarova et al., "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?," *The CRISPR Journal*, Vol. 1. No. 5, 2018, the entire contents of which are incorporated herein by reference. The particular CRISPR-Cas nomenclature used in any given instance in this Application is not limiting in any way and the skilled person will be able to identify which CRISPR-Cas enzyme is being referenced.

[0298] For example, the following type II, type V, and type VI Class 2 CRISPR-Cas enzymes have the following art-recognized old (i.e., legacy) and new names. Each of these enzymes, and/or variants thereof, may be used with the prime editors described herein:

Legacy nomenclature	Current nomenclature*
type II CRISPR-Cas enzymes	
Cas9	same
type V CRISPR-Cas enzymes	
Cpf1	Cas12a
CasX	Cas12e
C2c1	Cas12b1
Cas12b2	same
C2c3	Cas12c
CasY	Cas12d
C2c4	same
C2c8	same
C2c5	same
C2c10	same
C2c9	same
type VI CRISPR-Cas enzymes	
C2c2	Cas13a
Cas13d	same
C2c7	Cas13c
C2c6	Cas13b

* See Makarova et al., *The CRISPR Journal*, Vol. 1, No. 5, 2018

[0299] Without being bound by theory, the mechanism of action of certain napDNAbp contemplated herein includes the step of forming an R-loop whereby the napDNAbp induces the unwinding of a double-strand DNA target, thereby separating the strands in the region bound by the napDNAbp. The guide RNA spacer then hybridizes to the "target strand" at the protospacer sequence. This displaces a "non-target strand" that is complementary to the target strand, which forms the single strand region of the R-loop. In some embodiments, the napDNAbp includes one or more nuclease activities, which then cut the DNA leaving various types of lesions. For example, the napDNAbp may comprises a nuclease activity that cuts the non-target strand at a first location, and/ or cuts the target strand at a second location. Depending on the nuclease activity, the target DNA can be cut to form a "double-stranded break" whereby both strands are cut. In other embodiments, the target DNA can be cut at only a single site, i.e., the DNA is "nicked" on one strand. Exemplary napDNAbp with different nuclease activities include "Cas9 nickase" ("nCas9") and a deactivated Cas9 having no nuclease activities ("dead Cas9" or "dCas9").

[0300] The below description of various napDNAbps which can be used in connection with the presently disclose prime editors is not meant to be limiting in any way. The prime editors may comprise the canonical SpCas9, or any ortholog Cas9 protein, or any variant Cas9 protein—including any naturally occurring variant, mutant, or otherwise engineered version

of Cas9—that is known or which can be made or evolved through a directed evolutionary or otherwise mutagenic process. In various embodiments, the Cas9 or Cas9 variants have a nickase activity, i.e., only cleave of strand of the target DNA sequence. In other embodiments, the Cas9 or Cas9 variants have inactive nucleases, i.e., are "dead" Cas9 proteins. Other variant Cas9 proteins that may be used are those having a smaller molecular weight than the canonical SpCas9 (e.g., for easier delivery) or having modified or rearranged primary amino acid structure (e.g., the circular permutant formats).

[0301] The prime editors described herein may also comprise Cas9 equivalents, including Cas12a (Cpf1) and Cas12b1 proteins which are the result of convergent evolution. The napDNAbps used herein (e.g., SpCas9, Cas9 variant, or Cas9 equivalents) may also may also contain various modifications that alter/enhance their PAM specifities. Lastly, the application contemplates any Cas9, Cas9 variant, or Cas9 equivalent which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% sequence identity to a reference Cas9 sequence, such as a references SpCas9 canonical sequence or a reference Cas9 equivalent (e.g., Cas12a (Cpf1)).

[0302] The napDNAbp can be a CRISPR (clustered regularly interspaced short palindromic repeat)-associated nuclease. As outlined above, CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3′-5′ exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gRNA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, e.g., Jinek M. et al., Science 337:816-821(2012), the entire contents of which is hereby incorporated by reference.

[0303] In some embodiments, the napDNAbp directs cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. In some embodiments, the napDNAbp directs cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence. In some embodiments, a vector encodes a napDNAbp that is mutated to with respect to a corresponding wild-type enzyme such that the mutated napDNAbp lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, H840A, N854A, and N863A in reference to the canonical SpCas9 sequence, or to equivalent amino acid positions in other Cas9 variants or Cas9 equivalents.

[0304] As used herein, the term "Cas protein" refers to a full-length Cas protein obtained from nature, a recombinant Cas protein having a sequences that differs from a naturally occurring Cas protein, or any fragment of a Cas protein that nevertheless retains all or a significant amount of the requisite basic functions needed for the disclosed methods, i.e., (i) possession of nucleic-acid programmable binding of the Cas protein to a target DNA, and (ii) ability to nick the target DNA sequence on one strand. The Cas proteins contemplated herein embrace CRISPR Cas 9 proteins, as well as Cas9 equivalents, variants (e.g., Cas9 nickase (nCas9) or nuclease inactive Cas9 (dCas9)) homologs, orthologs, or paralogs, whether naturally occurring or non-naturally occurring (e.g., engineered or recombinant), and may include a Cas9 equivalent from anyClass 2 CRISPR system (e.g., type II, V, VI), including Cas12a (Cpf1), Cas12e (CasX), Cas12b1 (C2c1), Cas12b2, Cas12c (C2c3), C2c4, C2c8, C2c5, C2c10, C2c9 Cas13a (C2c2), Cas13d, Cas13c (C2c7), Cas13b (C2c6), and Cas13b. Further Cas-equivalents are described in Makarova et al., "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector," Science 2016; 353(6299) and Makarova et al., "Classification and Nomenclature of CRISPR-Cas Systems: Where from Here?," *The CRISPR Journal*, Vol. 1. No. 5, 2018, the contents of which are incorporated herein by reference.

[0305] The terms "Cas9" or "Cas9 nuclease" or "Cas9 moiety" or "Cas9 domain" embrace any naturally occurring Cas9 from any organism, any naturally-occurring Cas9 equivalent or functional fragment thereof, any Cas9 homolog, ortholog, or paralog from any organism, and

any mutant or variant of a Cas9, naturally-occurring or engineered. The term Cas9 is not meant to be particularly limiting and may be referred to as a "Cas9 or equivalent." Exemplary Cas9 proteins are further described herein and/or are described in the art and are incorporated herein by reference. The present disclosure is unlimited with regard to the particular Cas9 that is employed in the prime editor (PE) of the invention.

[0306] As noted herein, Cas9 nuclease sequences and structures are well known to those of skill in the art (see, e.g., "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti et al., J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., *Proc. Natl. Acad. Sci.* U.S.A. 98:4658-4663(2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607(2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. *Science* 337:816-821(2012), the entire contents of each of which are incorporated herein by reference).

[0307] Examples of Cas9 and Cas9 equivalents are provided as follows; however, these specific examples are not meant to be limiting. The primer editor of the present disclosure may use any suitable napDNAbp, including any suitable Cas9 or Cas9 equivalent.

A. <u>Wild type canonical SpCas9</u>

[0308] In one embodiment, the primer editor constructs described herein may comprise the "canonical SpCas9" nuclease from *S. pyogenes*, which has been widely used as a tool for genome engineering and is categorized as the type II subgroup of enzymes of the Class 2 CRISPR-Cas systems. This Cas9 protein is a large, multi-domain protein containing two distinct nuclease domains. Point mutations can be introduced into Cas9 to abolish one or both nuclease activities, resulting in a nickase Cas9 (nCas9) or dead Cas9 (dCas9), respectively, that still retains its ability to bind DNA in a sgRNA-programmed manner. In principle, when fused to another protein or domain, Cas9 or variant thereof (e.g., nCas9) can target that protein to virtually any DNA sequence simply by co-expression with an appropriate sgRNA. As used herein, the canonical SpCas9 protein refers to the wild type protein from *Streptococcus pyogenes* having the following amino acid sequence:

Description	Sequence	SEQ ID NO:

SpCas9 *Streptococcus pyogenes* M1 SwissProt Accession No. Q99ZW2 Wild type	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDI LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH AILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGM RKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEE NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEV VKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHD AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPT VAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEK NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPED NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDT TIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 18
SpCas9 Reverse translation of SwissProt Accession No. Q99ZW2 *Streptococcus pyogenes*	ATGGATAAAAAATATAGCATTGGCCTGGATATTGGC ACCAACAGCGTGGGCTGGGCGGTGATTACCGATGAA TATAAAGTGCCGAGCAAAAAATTTAAAGTGCTGGGC AACACCGATCGCCATAGCATTAAAAAAAACCTGATT GGCGCGCTGCTGTTTGATAGCGGCGAAACCGCGGAA GCGACCCGCCTGAAACGCACCGCGCGCCGCCGCTAT ACCCGCCGCAAAAACCGCATTTGCTATCTGCAGGAA ATTTTTAGCAACGAAATGGCGAAAGTGGATGATAGC TTTTTTCATCGCCTGGAAGAAAGCTTTCTGGTGGAAG AAGATAAAAAACATGAACGCCATCCGATTTTTGGCA ACATTGTGGATGAAGTGGCGTATCATGAAAAATATC CGACCATTTATCATCTGCGCAAAAAACTGGTGGATA GCACCGATAAAGCGGATCTGCGCCTGATTTATCTGG	SEQ ID NO: 19

	CGCTGGCGCATATGATTAAATTTCGCGGCCATTTTCT GATTGAAGGCGATCTGAACCCGGATAACAGCGATGT GGATAAACTGTTTATTCAGCTGGTGCAGACCTATAA CCAGCTGTTTGAAGAAAACCCGATTAACGCGAGCGG CGTGGATGCGAAAGCGATTCTGAGCGCGCGCCTGAG CAAAAGCCGCCGCCTGGAAAACCTGATTGCGCAGCT GCCGGGCGAAAAAAAAAAACGGCCTGTTTGGCAACCT GATTGCGCTGAGCCTGGGCCTGACCCCGAACTTTAA AAGCAACTTTGATCTGGCGGAAGATGCGAAACTGCA GCTGAGCAAAGATACCTATGATGATGATCTGGATAA CCTGCTGGCGCAGATTGGCGATCAGTATGCGGATCT GTTTCTGGCGGCGAAAAACCTGAGCGATGCGATTCT GCTGAGCGATATTCTGCGCGTGAACACCGAAATTAC CAAAGCGCCGCTGAGCGCGAGCATGATTAAACGCTA TGATGAACATCATCAGGATCTGACCCTGCTGAAAGC GCTGGTGCGCCAGCAGCTGCCGGAAAAATATAAAG AAATTTTTTTTGATCAGAGCAAAAACGGCTATGCGG GCTATATTGATGGCGGCGCGAGCCAGGAAGAATTTT ATAAATTTATTAAACCGATTCTGGAAAAAATGGATG GCACCGAAGAACTGCTGGTGAAACTGAACCGCGAA GATCTGCTGCGCAAACAGCGCACCTTTGATAACGGC AGCATTCCGCATCAGATTCATCTGGGCGAACTGCAT GCGATTCTGCGCCGCCAGGAAGATTTTTATCCGTTTC TGAAAGATAACCGCGAAAAAATTGAAAAAATTCTG ACCTTTCGCATTCCGTATTATGTGGGCCCGCTGGCGC GCGGCAACAGCCGCTTTGCGTGGATGACCCGCAAAA GCGAAGAAACCATTACCCCGTGGAACTTTGAAGAAG TGGTGGATAAAGGCGCGAGCGCGCAGAGCTTTATTG AACGCATGACCAACTTTGATAAAAACCTGCCGAACG AAAAAGTGCTGCCGAAACATAGCCTGCTGTATGAAT ATTTTACCGTGTATAACGAACTGACCAAAGTGAAAT ATGTGACCGAAGGCATGCGCAAACCGGCGTTTCTGA GCGGCGAACAGAAAAAAGCGATTGTGGATCTGCTGT TTAAAACCAACCGCAAAGTGACCGTGAAACAGCTGA AAGAAGATTATTTTAAAAAAATTGAATGCTTTGATA GCGTGGAAATTAGCGGCGTGGAAGATCGCTTTAACG CGAGCCTGGGCACCTATCATGATCTGCTGAAAATTA TTAAAGATAAAGATTTTCTGGATAACGAAGAAAACG AAGATATTCTGGAAGATATTGTGCTGACCCTGACCC TGTTTGAAGATCGCGAAATGATTGAAGAACGCCTGA AAACCTATGCGCATCTGTTTGATGATAAAGTGATGA AACAGCTGAAACGCCGCCGCTATACCGGCTGGGGCC GCCTGAGCCGCAAACTGATTAACGGCATTCGCGATA AACAGAGCGGCAAAACCATTCTGGATTTTCTGAAAA GCGATGGCTTTGCGAACCGCAACTTTATGCAGCTGA TTCATGATGATAGCCTGACCTTTAAAGAAGATATTC AGAAAGCGCAGGTGAGCGGCCAGGGCGATAGCCTG CATGAACATATTGCGAACCTGGCGGGCAGCCCGGCG ATTAAAAAAGGCATTCTGCAGACCGTGAAAGTGGTG	

	GATGAACTGGTGAAAGTGATGGGCCGCCATAAACCG GAAAACATTGTGATTGAAATGGCGCGCGAAAACCA GACCACCCAGAAAGGCCAGAAAAACAGCCGCGAAC GCATGAAACGCATTGAAGAAGGCATTAAAGAACTG GGCAGCCAGATTCTGAAAGAACATCCGGTGGAAAA CACCCAGCTGCAGAACGAAAAACTGTATCTGTATTA TCTGCAGAACGGCCGCGATATGTATGTGGATCAGGA ACTGGATATTAACCGCCTGAGCGATTATGATGTGGA TCATATTGTGCCGCAGAGCTTTCTGAAAGATGATAG CATTGATAACAAAGTGCTGACCCGCAGCGATAAAAA CCGCGGCAAAAGCGATAACGTGCCGAGCGAAGAAG TGGTGAAAAAAATGAAAAACTATTGGCGCCAGCTGC TGAACGCGAAACTGATTACCCAGCGCAAATTTGATA ACCTGACCAAAGCGGAACGCGGCGGCCTGAGCGAA CTGGATAAAGCGGGCTTTATTAAACGCCAGCTGGTG GAAACCCGCCAGATTACCAAACATGTGGCGCAGATT CTGGATAGCCGCATGAACACCAAATATGATGAAAAC GATAAACTGATTCGCGAAGTGAAAGTGATTACCCTG AAAAGCAAACTGGTGAGCGATTTTCGCAAAGATTTT CAGTTTTATAAAGTGCGCGAAATTAACAACTATCAT CATGCGCATGATGCGTATCTGAACGCGGTGGTGGGC ACCGCGCTGATTAAAAAATATCCGAAACTGGAAAGC GAATTTGTGTATGGCGATTATAAAGTGTATGATGTG CGCAAAATGATTGCGAAAAGCGAACAGGAAATTGG CAAAGCGACCGCGAAATATTTTTTTTATAGCAACAT TATGAACTTTTTTAAAACCGAAATTACCCTGGCGAA CGGCGAAATTCGCAAACGCCCGCTGATTGAAACCAA CGGCGAAACCGGCGAAATTGTGTGGGATAAAGGCC GCGATTTTGCGACCGTGCGCAAAGTGCTGAGCATGC CGCAGGTGAACATTGTGAAAAAAACCGAAGTGCAG ACCGGCGGCTTTAGCAAAGAAAGCATTCTGCCGAAA CGCAACAGCGATAAACTGATTGCGCGCAAAAAAGA TTGGGATCCGAAAAAATATGGCGGCTTTGATAGCCC GACCGTGGCGTATAGCGTGCTGGTGGTGGCGAAAGT GGAAAAAGGCAAAAGCAAAAAACTGAAAAGCGTGA AAGAACTGCTGGGCATTACCATTATGGAACGCAGCA GCTTTGAAAAAAACCCGATTGATTTTCTGGAAGCGA AAGGCTATAAAGAAGTGAAAAAAGATCTGATTATTA AACTGCCGAAATATAGCCTGTTTGAACTGGAAAACG GCCGCAAACGCATGCTGGCGAGCGCGGGCGAACTG CAGAAAGGCAACGAACTGGCGCTGCCGAGCAAATA TGTGAACTTTCTGTATCTGGCGAGCCATTATGAAAA ACTGAAAGGCAGCCCGGAAGATAACGAACAGAAAC AGCTGTTTGTGGAACAGCATAAACATTATCTGGATG AAATTATTGAACAGATTAGCGAATTTAGCAAACGCG TGATTCTGGCGGATGCGAACCTGGATAAAGTGCTGA GCGCGTATAACAAACATCGCGATAAACCGATTCGCG AACAGGCGGAAAACATTATTCATCTGTTTACCCTGA CCAACCTGGGCGCGCCGGCGGCGTTTAAATATTTTG	

	ATACCACCATTGATCGCAAACGCTATACCAGCACCA AAGAAGTGCTGGATGCGACCCTGATTCATCAGAGCA TTACCGGCCTGTATGAAACCCGCATTGATCTGAGCC AGCTGGGCGGCGAT	

[0309] The prime editors described herein may include canonical SpCas9, or any variant thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with a wild type Cas9 sequence provided above. These variants may include SpCas9 variants containing one or more mutations, including any known mutation reported with the SwissProt Accession No. Q99ZW2 (SEQ ID NO: 18) entry, which include:

SpCas9 mutation (relative to the amino acid sequence of the canonical SpCas9 sequence, SEQ ID NO: 18)	Function/Characteristic (as reported) (see UniProtKB – Q99ZW2 (CAS9_STRPT1) entry – incorporated herein by reference)
D10A	Nickase mutant which cleaves the protospacer strand (but no cleavage of non-protospacer strand)
S15A	Decreased DNA cleavage activity
R66A	Decreased DNA cleavage activity
R70A	No DNA cleavage
R74A	Decreased DNA cleavage
R78A	Decreased DNA cleavage
97-150 deletion	No nuclease activity
R165A	Decreased DNA cleavage
175-307 deletion	About 50% decreased DNA cleavage
312-409 deletion	No nuclease activity
E762A	Nickase
H840A	Nickase mutant which cleaves the non-protospacer strand but does not cleave the protospacer strand
N854A	Nickase
N863A	Nickase
H982A	Decreased DNA cleavage
D986A	Nickase
1099-1368 deletion	No nuclease activity
R1333A	Reduced DNA binding

[0310] Other wild type SpCas9 sequences that may be used in the present disclosure, include:

Description	Sequence	SEQ ID NO:
SpCas9 *Streptococcus pyogenes* MGAS1882 wild type NC_017053.1	ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCA CAAATAGCGTCGGATGGGCGGTGATCACTGATGATTA TAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAAT ACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGG CTCTTTTATTTGGCAGTGGAGAGACAGCGGAAGCGAC TCGTCTCAAACGGACAGCTCGTAGAAGGTATACACGT CGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATC GACTTGAAGAGTCTTTTTTGGTGGAAGAAGACAAGAA GCATGAACGTCATCCTATTTTTGGAAATATAGTAGATG AAGTTGCTTATCATGAGAAATATCCAACTATCTATCAT	SEQ ID NO: 20

	CTGCGAAAAAAATTGGCAGATTCTACTGATAAAGCGG ATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATT AAGTTTCGTGGTCATTTTTTGATTGAGGGAGATTTAAA TCCTGATAATAGTGATGTGGACAAACTATTTATCCAGT TGGTACAAATCTACAATCAATTATTTGAAGAAAACCCT ATTAACGCAAGTAGAGTAGATGCTAAAGCGATTCTTTC TGCACGATTGAGTAAATCAAGACGATTAGAAAATCTC ATTGCTCAGCTCCCCGGTGAGAAGAGAAATGGCTTGTT TGGGAATCTCATTGCTTTGTCATTGGGATTGACCCCTA ATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAA TTACAGCTTTCAAAAGATACTTACGATGATGATTTAGA TAATTTATTGGCGCAAATTGGAGATCAATATGCTGATT TGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTA CTTTCAGATATCCTAAGAGTAAATAGTGAAATAACTA AGGCTCCCCTATCAGCTTCAATGATTAAGCGCTACGAT GAACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGT TCGACAACAACTTCCAGAAAAGTATAAAGAAATCTTT TTTGATCAATCAAAAAACGGATATGCAGGTTATATTGA TGGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATC AAACCAATTTTAGAAAAAATGGATGGTACTGAGGAAT TATTGGTGAAACTAAATCGTGAAGATTTGCTGCGCAA GCAACGGACCTTTGACAACGGCTCTATTCCCCATCAAA TTCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAA GAAGACTTTTATCCATTTTTAAAAGACAATCGTGAGAA GATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATG TTGGTCCATTGGCGCGTGGCAATAGTCGTTTTGCATGG ATGACTCGGAAGTCTGAAGAAACAATTACCCCATGGA ATTTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAA TCATTTATTGAACGCATGACAAACTTTGATAAAAATCT TCCAAATGAAAAAGTACTACCAAAACATAGTTTGCTTT ATGAGTATTTTACGGTTTATAACGAATTGACAAAGGTC AAATATGTTACTGAGGGAATGCGAAAACCAGCATTTC TTTCAGGTGAACAGAAGAAAGCCATTGTTGATTTACTC TTCAAAACAAATCGAAAAGTAACCGTTAAGCAATTAA AAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAG TGTTGAAATTTCAGGAGTTGAAGATAGATTTAATGCTT CATTAGGCGCCTACCATGATTTGCTAAAAATTATTAAA GATAAAGATTTTTTGGATAATGAAGAAAATGAAGATA TCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAA GATAGGGGGATGATTGAGGAAAGACTTAAAACATATG CTCACCTCTTTGATGATAAGGTGATGAAACAGCTTAAA CGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAA ATTGATTAATGGTATTAGGGATAAGCAATCTGGCAAA ACAATATTAGATTTTTTGAAATCAGATGGTTTTGCCAA TCGCAATTTTATGCAGCTGATCCATGATGATAGTTTGA CATTTAAAGAAGATATTCAAAAAGCACAGGTGTCTGG ACAAGGCCATAGTTTACATGAACAGATTGCTAACTTA GCTGGCAGTCCTGCTATTAAAAAAGGTATTTTACAGAC TGTAAAAATTGTTGATGAACTGGTCAAAGTAATGGGG	

	CATAAGCCAGAAAATATCGTTATTGAAATGGCACGTG AAAATCAGACAACTCAAAAGGGCCAGAAAAATTCGCG AGAGCGTATGAAACGAATCGAAGAAGGTATCAAAGA ATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAA AATACTCAATTGCAAAATGAAAAGCTCTATCTCTATTA TCTACAAAATGGAAGAGACATGTATGTGGACCAAGAA TTAGATATTAATCGTTTAAGTGATTATGATGTCGATCA CATTGTTCCACAAAGTTTCATTAAAGACGATTCAATAG ACAATAAGGTACTAACGCGTTCTGATAAAAATCGTGG TAAATCGGATAACGTTCCAAGTGAAGAAGTAGTCAAA AAGATGAAAAACTATTGGAGACAACTTCTAAACGCCA AGTTAATCACTCAACGTAAGTTTGATAATTTAACGAAA GCTGAACGTGGAGGTTTGAGTGAACTTGATAAAGCTG GTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAATC ACTAAGCATGTGGCACAAATTTTGGATAGTCGCATGA ATACTAAATACGATGAAAATGATAAACTTATTCGAGA GGTTAAAGTGATTACCTTAAAATCTAAATTAGTTTCTG ACTTCCGAAAAGATTTCCAATTCTATAAAGTACGTGAG ATTAACAATTACCATCATGCCCATGATGCGTATCTAAA TGCCGTCGTTGGAACTGCTTTGATTAAGAAATATCCAA AACTTGAATCGGAGTTTGTCTATGGTGATTATAAAGTT TATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGA AATAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTA ATATCATGAACTTCTTCAAAACAGAAATTACACTTGCA AATGGAGAGATTCGCAAACGCCCTCTAATCGAAACTA ATGGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCG AGATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCC AAGTCAATATTGTCAAGAAAACAGAAGTACAGACAGG CGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAAT TCGGACAAGCTTATTGCTCGTAAAAAAGACTGGGATC CAAAAAAATATGGTGGTTTTGATAGTCCAACGGTAGC TTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGG AAATCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAG GGATCACAATTATGGAAAGAAGTTCCTTTGAAAAAAA TCCGATTGACTTTTTAGAAGCTAAAGGATATAAGGAA GTTAAAAAAGACTTAATCATTAAACTACCTAAATATA GTCTTTTTGAGTTAGAAAACGGTCGTAAACGGATGCTG GCTAGTGCCGGAGAATTACAAAAAGGAAATGAGCTGG CTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCT AGTCATTATGAAAAGTTGAAGGGTAGTCCAGAAGATA ACGAACAAAAACAATTGTTTGTGGAGCAGCATAAGCA TTATTTAGATGAGATTATTGAGCAAATCAGTGAATTTT CTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAA GTTCTTAGTGCATATAACAAACATAGAGACAAACCAA TACGTGAACAAGCAGAAAATATTATTCATTTATTTACG TTGACGAATCTTGGAGCTCCCGCTGCTTTTAAATATTT TGATACAACAATTGATCGTAAACGATATACGTCTACA AAAGAAGTTTTAGATGCCACTCTTATCCATCAATCCAT	

	CACTGGTCTTTATGAAACACGCATTGATTTGAGTCAGC TAGGAGGTGACTGA	
SpCas9 *Streptococcus pyogenes* MGAS1882 wild type NC_017053.1	MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNT DRHSIKKNLIGALLFGSGETAEATRLKRTARRRYTRRKN RICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHP IFGNIVDEVAYHEKYPTIYHLRKKLADSTDKADLRLIYLA LAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQIYNQLFE ENPINASRVDAKAILSARLSKSRRLENLIAQLPGEKRNGL FGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLD NLLAQIGDQYADLFLAAKNLSDAILLSDILRVNSEITKAP LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQS KNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITP WNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLL YEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLG AYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDRGMIE ERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIR DKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKA QVSGQGHSLHEQIANLAGSPAIKKGILQTVKIVDELVKV MGHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFIKDDSIDNKVLTRSDKNRGKSD NVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERG GLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDE NDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHA HDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQ TGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVA YSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPID FLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGE LQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLF VEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 21
SpCas9 *Streptococcus pyogenes* wild type SWBC2D7W014	ATGGATAAAAAGTATTCTATTGGTTTAGACATCGGCAC TAATTCCGTTGGATGGGCTGTCATAACCGATGAATACA AAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACAC AGACCGTCATTCGATTAAAAAGAATCTTATCGGTGCCC TCCTATTCGATAGTGGCGAAACGGCAGAGGCGACTCG CCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGC AAGAACCGAATATGTTACTTACAAGAAATTTTTAGCA ATGAGATGGCCAAAGTTGACGATTCTTTCTTTCACCGT TTGGAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAAC ATGAACGGCACCCCATCTTTGGAAACATAGTAGATGA GGTGGCATATCATGAAAAGTACCCAACGATTTATCAC CTCAGAAAAAAGCTAGTTGACTCAACTGATAAAGCGG	SEQ ID NO: 22

	ACCTGAGGTTAATCTACTTGGCTCTTGCCCATATGATA AAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAA TCCGGACAACTCGGATGTCGACAAACTGTTCATCCAGT TAGTACAAACCTATAATCAGTTGTTTGAAGAGAACCCT ATAAATGCAAGTGGCGTGGATGCGAAGGCTATTCTTA GCGCCCGCCTCTCTAAATCCCGACGGCTAGAAAACCT GATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTG TTCGGTAACCTTATAGCGCTCTCACTAGGCCTGACACC AAATTTTAAGTCGAACTTCGACTTAGCTGAAGATGCCA AATTGCAGCTTAGTAAGGACACGTACGATGACGATCT CGACAATCTACTGGCACAAATTGGAGATCAGTATGCG GACTTATTTTTGGCTGCCAAAAACCTTAGCGATGCAAT CCTCCTATCTGACATACTGAGAGTTAATACTGAGATTA CCAAGGCGCCGTTATCCGCTTCAATGATCAAAAGGTA CGATGAACATCACCAAGACTTGACACTTCTCAAGGCC CTAGTCCGTCAGCAACTGCCTGAGAAATATAAGGAAA TATTCTTTGATCAGTCGAAAAACGGGTACGCAGGTTAT ATTGACGGCGGAGCGAGTCAAGAGGAATTCTACAAGT TTATCAAACCCATATTAGAGAAGATGGATGGGACGGA AGAGTTGCTTGTAAAACTCAATCGCGAAGATCTACTGC GAAAGCAGCGGACTTTCGACAACGGTAGCATTCCACA TCAAATCCACTTAGGCGAATTGCATGCTATACTTAGAA GGCAGGAGGATTTTTATCCGTTCCTCAAAGACAATCGT GAAAAGATTGAGAAAATCCTAACCTTTCGCATACCTTA CTATGTGGGACCCCTGGCCCGAGGGAACTCTCGGTTCG CATGGATGACAAGAAAGTCCGAAGAAACGATTACTCC ATGGAATTTTGAGGAAGTTGTCGATAAAGGTGCGTCA GCTCAATCGTTCATCGAGAGGATGACCAACTTTGACA AGAATTTACCGAACGAAAAAGTATTGCCTAAGCACAG TTTACTTTACGAGTATTTCACAGTGTACAATGAACTCA CGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACC CGCCTTTCTAAGCGGAGAACAGAAGAAAGCAATAGTA GATCTGTTATTCAAGACCAACCGCAAAGTGACAGTTA AGCAATTGAAAGAGGACTACTTTAAGAAAATTGAATG CTTCGATTCTGTCGAGATCTCCGGGGTAGAAGATCGAT TTAATGCGTCACTTGGTACGTATCATGACCTCCTAAAG ATAATTAAAGATAAGGACTTCCTGGATAACGAAGAGA ATGAAGATATCTTAGAAGATATAGTGTTGACTCTTACC CTCTTTGAAGATCGGGAAATGATTGAGGAAAGACTAA AAACATACGCTCACCTGTTCGACGATAAGGTTATGAA ACAGTTAAAGAGGCGTCGCTATACGGGCTGGGGACGA TTGTCGCGGAAACTTATCAACGGGATAAGAGACAAGC AAAGTGGTAAAACTATTCTCGATTTTCTAAAGAGCGAC GGCTTCGCCAATAGGAACTTTATGCAGCTGATCCATGA TGACTCTTTAACCTTCAAAGAGGATATACAAAAGGCA CAGGTTTCCGGACAAGGGGACTCATTGCACGAACATA TTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGC ATACTCCAGACAGTCAAAGTAGTGGATGAGCTAGTTA AGGTCATGGGACGTCACAAACCGGAAAACATTGTAAT	

	CGAGATGGCACGCGAAAATCAAACGACTCAGAAGGG GCAAAAAAACAGTCGAGAGCGGATGAAGAGAATAGA AGAGGGTATTAAAGAACTGGGCAGCCAGATCTTAAAG GAGCATCCTGTGGAAAATACCCAATTGCAGAACGAGA AACTTTACCTCTATTACCTACAAAATGGAAGGGACATG TATGTTGATCAGGAACTGGACATAAACCGTTTATCTGA TTACGACGTCGATCACATTGTACCCCAATCCTTTTTGA AGGACGATTCAATCGACAATAAAGTGCTTACACGCTC GGATAAGAACCGAGGGAAAAGTGACAATGTTCCAAGC GAGGAAGTCGTAAAGAAAATGAAGAACTATTGGCGGC AGCTCCTAAATGCGAAACTGATAACGCAAAGAAAGTT CGATAACTTAACTAAAGCTGAGAGGGGTGGCTTGTCT GAACTTGACAAGGCCGGATTTATTAAACGTCAGCTCGT GGAAACCCGCCAAATCACAAAGCATGTTGCACAGATA CTAGATTCCCGAATGAATACGAAATACGACGAGAACG ATAAGCTGATTCGGGAAGTCAAAGTAATCACTTTAAA GTCAAAATTGGTGTCGGACTTCAGAAAGGATTTTCAAT TCTATAAAGTTAGGGAGATAAATAACTACCACCATGC GCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCAC TCATTAAGAAATACCCGAAGCTAGAAAGTGAGTTTGT GTATGGTGATTACAAAGTTTATGACGTCCGTAAGATGA TCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAG CCAAATACTTCTTTTATTCTAACATTATGAATTTCTTTA AGACGGAAATCACTCTGGCAAACGGAGAGATACGCAA ACGACCTTTAATTGAAACCAATGGGGAGACAGGTGAA ATCGTATGGGATAAGGGCCGGGACTTCGCGACGGTGA GAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAA GAAAACTGAGGTGCAGACCGGAGGGTTTTCAAAGGAA TCGATTCTTCCAAAAAGGAATAGTGATAAGCTCATCGC TCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGC TTCGATAGCCCTACAGTTGCCTATTCTGTCCTAGTAGT GGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGAA GTCAGTCAAAGAATTATTGGGGATAACGATTATGGAG CGCTCGTCTTTTGAAAAGAACCCCATCGACTTCCTTGA GGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATA ATTAAACTACCAAAGTATAGTCTGTTTGAGTTAGAAAA TGGCCGAAAACGGATGTTGGCTAGCGCCGGAGAGCTT CAAAAGGGGAACGAACTCGCACTACCGTCTAAATACG TGAATTTCCTGTATTTAGCGTCCCATTACGAGAAGTTG AAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTT TTGTTGAGCAGCACAAACATTATCTCGACGAAATCATA GAGCAAATTTCGGAATTCAGTAAGAGAGTCATCCTAG CTGATGCCAATCTGGACAAAGTATTAAGCGCATACAA CAAGCACAGGGATAAACCCATACGTGAGCAGGCGGAA AATATTATCCATTTGTTTACTCTTACCAACCTCGGCGCT CCAGCCGCATTCAAGTATTTTGACACAACGATAGATCG CAAACGATACACTTCTACCAAGGAGGTGCTAGACGCG ACACTGATTCACCAATCCATCACGGGATTATATGAAAC TCGGATAGATTTGTCACAGCTTGGGGGTGACGGATCCC	

	CCAAGAAGAAGAGGAAAGTCTCGAGCGACTACAAAG ACCATGACGGTGATTATAAAGATCATGACATCGATTA CAAGGATGACGATGACAAGGCTGCAGGA	
SpCas9 *Streptococcus pyogenes* wild type Encoded product of SWBC2D7W014	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGDGSPKKKRKVS SDYKDHDGDYKDHDIDYKDDDDKAAG	SEQ ID NO: 23
SpCas9 *Streptococcus pyogenes* M1GAS wild type NC_002737.2	ATGGATAAGAAATACTCAATAGGCTTAGATATCGGCA CAAATAGCGTCGGATGGGCGGTGATCACTGATGAATA TAAGGTTCCGTCTAAAAAGTTCAAGGTTCTGGGAAAT ACAGACCGCCACAGTATCAAAAAAAATCTTATAGGGG CTCTTTTATTTGACAGTGGAGAGACAGCGGAAGCGAC TCGTCTCAAACGGACAGCTCGTAGAAGGTATACACGT CGGAAGAATCGTATTTGTTATCTACAGGAGATTTTTTC AAATGAGATGGCGAAAGTAGATGATAGTTTCTTTCATC GACTTGAAGAGTCTTTTTTGGTGGAAGAAGACAAGAA GCATGAACGTCATCCTATTTTTGGAAATATAGTAGATG	SEQ ID NO: 24

	AAGTTGCTTATCATGAGAAATATCCAACTATCTATCAT CTGCGAAAAAAATTGGTAGATTCTACTGATAAAGCGG ATTTGCGCTTAATCTATTTGGCCTTAGCGCATATGATT AAGTTTCGTGGTCATTTTTTGATTGAGGGAGATTTAAA TCCTGATAATAGTGATGTGGACAAACTATTTATCCAGT TGGTACAAACCTACAATCAATTATTTGAAGAAAACCCT ATTAACGCAAGTGGAGTAGATGCTAAAGCGATTCTTTC TGCACGATTGAGTAAATCAAGACGATTAGAAAATCTC ATTGCTCAGCTCCCCGGTGAGAAGAAAAATGGCTTATT TGGGAATCTCATTGCTTTGTCATTGGGTTTGACCCCTA ATTTTAAATCAAATTTTGATTTGGCAGAAGATGCTAAA TTACAGCTTTCAAAAGATACTTACGATGATGATTTAGA TAATTTATTGGCGCAAATTGGAGATCAATATGCTGATT TGTTTTTGGCAGCTAAGAATTTATCAGATGCTATTTTA CTTTCAGATATCCTAAGAGTAAATACTGAAATAACTAA GGCTCCCCTATCAGCTTCAATGATTAAACGCTACGATG AACATCATCAAGACTTGACTCTTTTAAAAGCTTTAGTT CGACAACAACTTCCAGAAAAGTATAAAGAAATCTTTT TTGATCAATCAAAAAACGGATATGCAGGTTATATTGAT GGGGGAGCTAGCCAAGAAGAATTTTATAAATTTATCA AACCAATTTTAGAAAAAATGGATGGTACTGAGGAATT ATTGGTGAAACTAAATCGTGAAGATTTGCTGCGCAAG CAACGGACCTTTGACAACGGCTCTATTCCCCATCAAAT TCACTTGGGTGAGCTGCATGCTATTTTGAGAAGACAAG AAGACTTTTATCCATTTTTAAAAGACAATCGTGAGAAG ATTGAAAAAATCTTGACTTTTCGAATTCCTTATTATGTT GGTCCATTGGCGCGTGGCAATAGTCGTTTTGCATGGAT GACTCGGAAGTCTGAAGAAACAATTACCCCATGGAAT TTTGAAGAAGTTGTCGATAAAGGTGCTTCAGCTCAATC ATTTATTGAACGCATGACAAACTTTGATAAAAATCTTC CAAATGAAAAAGTACTACCAAAACATAGTTTGCTTTAT GAGTATTTTACGGTTTATAACGAATTGACAAAGGTCAA ATATGTTACTGAAGGAATGCGAAAACCAGCATTTCTTT CAGGTGAACAGAAGAAAGCCATTGTTGATTTACTCTTC AAAACAAATCGAAAAGTAACCGTTAAGCAATTAAAAG AAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGTT GAAATTTCAGGAGTTGAAGATAGATTTAATGCTTCATT AGGTACCTACCATGATTTGCTAAAAATTATTAAAGATA AAGATTTTTTGGATAATGAAGAAAATGAAGATATCTT AGAGGATATTGTTTTAACATTGACCTTATTTGAAGATA GGGAGATGATTGAGGAAAGACTTAAAACATATGCTCA CCTCTTTGATGATAAGGTGATGAAACAGCTTAAACGTC GCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAATTG ATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAA TATTAGATTTTTTGAAATCAGATGGTTTTGCCAATCGC AATTTTATGCAGCTGATCCATGATGATAGTTTGACATT TAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAA GGCGATAGTTTACATGAACATATTGCAAATTTAGCTGG TAGCCCTGCTATTAAAAAAGGTATTTTACAGACTGTAA	

	AAGTTGTTGATGAATTGGTCAAAGTAATGGGGCGGCA TAAGCCAGAAAATATCGTTATTGAAATGGCACGTGAA AATCAGACAACTCAAAAGGGCCAGAAAAATTCGCGAG AGCGTATGAAACGAATCGAAGAAGGTATCAAAGAATT AGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAAT ACTCAATTGCAAAATGAAAAGCTCTATCTCTATTATCT CCAAAATGGAAGAGACATGTATGTGGACCAAGAATTA GATATTAATCGTTTAAGTGATTATGATGTCGATCACAT TGTTCCACAAAGTTTCCTTAAAGACGATTCAATAGACA ATAAGGTCTTAACGCGTTCTGATAAAAATCGTGGTAA ATCGGATAACGTTCCAAGTGAAGAAGTAGTCAAAAAG ATGAAAAACTATTGGAGACAACTTCTAAACGCCAAGT TAATCACTCAACGTAAGTTTGATAATTTAACGAAAGCT GAACGTGGAGGTTTGAGTGAACTTGATAAAGCTGGTT TTATCAAACGCCAATTGGTTGAAACTCGCCAAATCACT AAGCATGTGGCACAAATTTTGGATAGTCGCATGAATA CTAAATACGATGAAAATGATAAACTTATTCGAGAGGT TAAAGTGATTACCTTAAAATCTAAATTAGTTTCTGACT TCCGAAAAGATTTCCAATTCTATAAAGTACGTGAGATT AACAATTACCATCATGCCCATGATGCGTATCTAAATGC CGTCGTTGGAACTGCTTTGATTAAGAAATATCCAAAAC TTGAATCGGAGTTTGTCTATGGTGATTATAAAGTTTAT GATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAA TAGGCAAAGCAACCGCAAAATATTTCTTTTACTCTAAT ATCATGAACTTCTTCAAAACAGAAATTACACTTGCAAA TGGAGAGATTCGCAAACGCCCTCTAATCGAAACTAAT GGGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAG ATTTTGCCACAGTGCGCAAAGTATTGTCCATGCCCCAA GTCAATATTGTCAAGAAAACAGAAGTACAGACAGGCG GATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTC GGACAAGCTTATTGCTCGTAAAAAAGACTGGGATCCA AAAAAATATGGTGGTTTTGATAGTCCAACGGTAGCTTA TTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAA TCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAGGGA TCACAATTATGGAAAGAAGTTCCTTTGAAAAAAATCC GATTGACTTTTTAGAAGCTAAAGGATATAAGGAAGTT AAAAAAGACTTAATCATTAAACTACCTAAATATAGTCT TTTTGAGTTAGAAAACGGTCGTAAACGGATGCTGGCT AGTGCCGGAGAATTACAAAAAGGAAATGAGCTGGCTC TGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACG AACAAAAACAATTGTTTGTGGAGCAGCATAAGCATTA TTTAGATGAGATTATTGAGCAAATCAGTGAATTTTCTA AGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTT CTTAGTGCATATAACAAACATAGAGACAAACCAATAC GTGAACAAGCAGAAAATATTATTCATTTATTTACGTTG ACGAATCTTGGAGCTCCCGCTGCTTTTAAATATTTTGA TACAACAATTGATCGTAAACGATATACGTCTACAAAA GAAGTTTTAGATGCCACTCTTATCCATCAATCCATCAC	

	TGGTCTTTATGAAACACGCATTGATTTGAGTCAGCTAG GAGGTGACTGA	
SpCas9 *Streptococcus pyogenes* M1GAS wild type Encoded product of NC_002737.2 (100% identical to the canonical Q99ZW2 wild type)	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 25

[0311] The prime editors described herein may include any of the above SpCas9 sequences, or any variant thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

B. Wild type Cas9 orthologs

[0312] In other embodiments, the Cas9 protein can be a wild type Cas9 ortholog from another bacterial species different from the canonical Cas9 from *S. pyogenes*. For example, the following Cas9 orthologs can be used in connection with the prime editor constructs described in this specification. In addition, any variant Cas9 orthologs having at least 80%, at

least 85%, at least 90%, at least 95%, or at least 99% sequence identity to any of the below orthologs may also be used with the present prime editors.

Description	Sequence
LfCas9 *Lactobacillus fermentum* wild type *GenBank: SNX31424.1* 1	MKEYHIGLDIGTSSIGWAVTDSQFKLMRIKGKTAIGVRLFEEGKTAAERR TFRTTRRRLKRRKWRLHYLDEIFAPHLQEVDENFLRRLKQSNIHPEDPTK NQAFIGKLLFPDLLKKNERGYPTLIKMRDELPVEQRAHYPVMNIYKLRE AMINEDRQFDLREVYLAVHHIVKYRGHFLNNASVDKFKVGRIDFDKSFN VLNEAYEELQNGEGSFTIEPSKVEKIGQLLLDTKMRKLDRQKAVAKLLE VKVADKEETKRNKQIATAMSKLVLGYKADFATVAMANGNEWKIDLSS ETSEDEIEKFREELSDAQNDILTEITSLFSQIMLNEIVPNGMSISESMMDRY WTHERQLAEVKEYLATQPASARKEFDQVYNKYIGQAPKERGFDLEKGL KKILSKKENWKEIDELLKAGDFLPKQRTSANGVIPHQMHQQELDRIIEKQ AKYYPWLATENPATGERDRHQAKYELDQLVSFRIPYYVGPLVTPEVQK ATSGAKFAWAKRKEDGEITPWNLWDKIDRAESAEAFIKRMTVKDTYLL NEDVLPANSLLYQKYNVLNELNNVRVNGRRLSVGIKQDIYTELFKKKKT VKASDVASLVMAKTRGVNKPSVEGLSDPKKFNSNLATYLDLKSIVGDK VDDNRYQTDLENIIEWRSVFEDGEIFADKLTEVEWLTDEQRSALVKKRY KGWGRLSKKLLTGIVDENGQRIIDLMWNTDQNFKEIVDQPVFKEQIDQL NQKAITNDGMTLRERVESVLDDAYTSPQNKKAIWQVVRVVEDIVKAVG NAPKSISIEFARNEGNKGEITRSRRTQLQKLFEDQAHELVKDTSLTEELEK APDLSDRYYFYFTQGGKDMYTGDPINFDEISTKYDIDHILPQSFVKDNSL DNRVLTSRKENNKKSDQVPAKLYAAKMKPYWNQLLKQGLITQRKFEN LTKDVDQNIKYRSLGFVKRQLVETRQVIKLTANILGSMYQEAGTEIIETR AGLTKQLREEFDLPKVREVNDYHHAVDAYLTTFAGQYLNRRYPKLRSF FVYGEYMKFKHGSDLKLRNFNFFHELMEGDKSQGKVVDQQTGELITTR DEVAKSFDRLLNMKYMLVSKEVHDRSDQLYGATIVTAKESGKLTSPIEI KKNRLVDLYGAYTNGTSAFMTIIKFTGNKPKYKVIGIPTTSAASLKRAGK PGSESYNQELHRIIKSNPKVKKGFEIVVPHVSYGQLIVDGDCKFTLASPTV QHPATQLVLSKKSLETISSGYKILKDKPAIANERLIRVFDEVVGQMNRYF TIFDQRSNRQKVADARDKFLSLPTESKYEGAKKVQVGKTEVITNLLMGL HANATQGDLKVLGLATFGFFQSTTGLSLSEDTMIVYQSPTGLFERRICLK DI (SEQ ID NO: 26)
SaCas9 *Staphylococcus aureus* wild type GenBank: AYD60528.1	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIG ALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFH RLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKA DLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEEN PINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSD AILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYK EIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFD KNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAI VDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVM KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLI HDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQIL

Description	Sequence
	KEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQ SFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI TQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNT KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLN AVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFY SNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPT VAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYK EVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLY LASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYT STKEVLDATLIHQSITGLYETRIDLSQLGGD (SEQ ID NO: 27)
SaCas9 *Staphylococcus aureus*	MGKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRS KRGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQ KLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEE KYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQ SFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELR SVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKP TLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENA ELLDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSL KAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSP VVKRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNR QTNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNP FNYEVDHIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKISY ETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRY ATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYK HHAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQ EYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRKLINDTLYSTRKDDKGN TLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQ YGDEKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDIT DDYPNSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEV NSKCYEEAKKLKKISNQAEFIASFYKNDLIKINGELYRVIGVNNDLLNRIE VNMIDITYREYLENMNDKRPPHIIKTIASKTQSIKKYSTDILGNLYEVKSK KHPQIIKK (SEQ ID NO: 28)
StCas9 *Streptococcus thermophilus* UniProtKB/Swiss-Prot: G3ECR1.2 Wild type	MLFNKCIIISINLDFSNKEKCMTKPYSIGLDIGTNSVGWAVITDNYKVPSK KMKVLGNTSKKYIKKNLLGVLLFDSGITAEGRRLKRTARRRYTRRRNRI LYLQEIFSTEMATLDDAFFQRLDDSFLVPDDKRDSKYPIFGNLVEEKVYH DEFPTIYHLRKYLADSTKKADLRLVYLALAHMIKYRGHFLIEGEFNSKN NDIQKNFQDFLDTYNAIFESDLSLENSKQLEEIVKDKISKLEKKDRILKLF PGEKNSGIFSEFLKLIVGNQADFRKCFNLDEKASLHFSKESYDEDLETLL GYIGDDYSDVFLKAKKLYDAILLSGFLTVTDNETEAPLSSAMIKRYNEH KEDLALLKEYIRNISLKTYNEVFKDDTKNGYAGYIDGKTNQEDFYVYLK NLLAEFEGADYFLEKIDREDFLRKQRTFDNGSIPYQIHLQEMRAILDKQA KFYPFLAKNKERIEKILTFRIPYYVGPLARGNSDFAWSIRKRNEKITPWNF EDVIDKESSAEAFINRMTSFDLYLPEEKVLPKHSLLYETFNVYNELTKVR FIAESMRDYQFLDSKQKKDIVRLYFKDKRKVTDKDIIEYLHAIYGYDGIE LKGIEKQFNSSLSTYHDLLNIINDKEFLDDSSNEAIIEEIIHTLTIFEDREMIK

Description	Sequence
	QRLSKFENIFDKSVLKKLSRRHYTGWGKLSAKLINGIRDEKSGNTILDYLI DDGISNRNFMQLIHDDALSFKKKIQKAQIIGDEDKGNIKEVVKSLPGSPAI KKGILQSIKIVDELVKVMGGRKPESIVVEMARENQYTNQGKSNSQQRLK RLEKSLKELGSKILKENIPAKLSKIDNNALQNDRLYLYYLQNGKDMYTG DDLDIDRLSNYDIDHIIPQAFLKDNSIDNKVLVSSASNRGKSDDFPSLEVV KKRKTFWYQLLKSKLISQRKFDNLTKAERGGLLPEDKAGFIQRQLVETR QITKHVARLLDEKFNNKKDENNRAVRTVKIITLKSTLVSQFRKDFELYK VREINDFHHAHDAYLNAVIASALLKKYPKLEPEFVYGDYPKYNSFRERK SATEKVYFYSNIMNIFKKSISLADGRVIERPLIEVNEETGESVWNKESDLA TVRRVLSYPQVNVVKKVEEQNHGLDRGKPKGLFNANLSSKPKPNSNEN LVGAKEYLDPKKYGGYAGISNSFAVLVKGTIEKGAKKKITNVLEFQGISI LDRINYRKDKLNFLLEKGYKDIELIIELPKYSLFELSDGSRRMLASILSTN NKRGEIHKGNQIFLSQKFVKLLYHAKRISNTINENHRKYVENHKKEFEEL FYYILEFNENYVGAKKNGKLLNSAFQSWQNHSIDELCSSFIGPTGSERKG LFELTSRGSAADFEFLGVKIPRYRDYTPSSLLKDATLIHQSVTGLYETRID LAKLGEG (SEQ ID NO: 29)
LcCas9 *Lactobacillus crispatus* NCBI Reference Sequence: WP_133478044.1 Wild type	MKIKNYNLALTPSTSAVGHVEVDDDLNILEPVHHQKAIGVAKFGEGETA EARRLARSARRTTKRRANRINHYFNEIMKPEIDKVDPLMFDRIKQAGLSP LDERKEFRTVIFDRPNIASYYHNQFPTIWHLQKYLMITDEKADIRLIYWA LHSLLKHRGHFFNTTPMSQFKPGKLNLKDDMLALDDYNDLEGLSFAVA NSPEIEKVIKDRSMHKKEKIAELKKLIVNDVPDKDLAKRNNKIITQIVNAI MGNSFHLNFIFDMDLDKLTSKAWSFKLDDPELDTKFDAISGSMTDNQIGI FETLQKIYSAISLLDILNGSSNVVDAKNALYDKHKRDLNLYFKFLNTLPD EIAKTLKAGYTLYIGNRKKDLLAARKLLKVNVAKNFSQDDFYKLINKEL KSIDKQGLQTRFSEKVGELVAQNNFLPVQRSSDNVFIPYQLNAITFNKILE NQGKYYDFLVKPNPAKKDRKNAPYELSQLMQFTIPYYVGPLVTPEEQV KSGIPKTSRFAWMVRKDNGAITPWNFYDKVDIEATADKFIKRSIAKDSY LLSELVLPKHSLLYEKYEVFNELSNVSLDGKKLSGGVKQILFNEVFKKTN KVNTSRILKALAKHNIPGSKITGLSNPEEFTSSLQTYNAWKKYFPNQIDNF AYQQDLEKMIEWSTVFEDHKILAKKLDEIEWLDDDQKKFVANTRLRGW GRLSKRLLTGLKDNYGKSIMQRLETTKANFQQIVYKPEFREQIDKISQAA AKNQSLEDILANSYTSPSNRKAIRKTMSVVDEYIKLNHGKEPDKIFLMFQ RSEQEKGKQTEARSKQLNRILSQLKADKSANKLFSKQLADEFSNAIKKS KYKLNDKQYFYFQQLGRDALTGEVIDYDELYKYTVLHIIPRSKLTDDSQ NNKVLTKYKIVDGSVALKFGNSYSDALGMPIKAFWTELNRLKLIPKGKL LNLTTDFSTLNKYQRDGYIARQLVETQQIVKLLATIMQSRFKHTKIIEVR NSQVANIRYQFDYFRIKNLNEYYRGFDAYLAAVVGTYLYKVYPKARRL FVYGQYLKPKKTNQENQDMHLDSEKKSQGFNFLWNLLYGKQDQIFVN GTDVIAFNRKDLITKMNTVYNYKSQKISLAIDYHNGAMFKATLFPRNDR DTAKTRKLIPKKKDYDTDIYGGYTSNVDGYMLLAEIIKRDGNKQYGFYG VPSRLVSELDTLKKTRYTEYEEKLKEIIKPELGVDLKKIKKIKILKNKVPF NQVIIDKGSKFFITSTSYRWNYRQLILSAESQQTLMDLVVDPDFSNHKAR KDARKNADERLIKVYEEILYQVKNYMPMFVELHRCYEKLVDAQKTFKS LKISDKAMVLNQILILLHSNATSPVLEKLGYHTRFTLGKKHNLISENAVL VTQSITGLKENHVSIKQML (SEQ ID NO: 30)
PdCas9 *Pedicoccus damnosus*	MTNEKYSIGLDIGTSSIGFAVVNDNNRVIRVKGKNAIGVRLFDEGKAAA DRRSFRTTRRSFRTTRRRLSRRRWRLKLLREIFDAYITPVDEAFFIRLKES NLSPKDSKKQYSGDILFNDRSDKDFYEKYPTIYHLRNALMTEHRKFDVR

Description	Sequence
NCBI Reference Sequence: WP_062913273.1 Wild type	EIYLAIHHIMKFRGHFLNATPANNFKVGRLNLEEKFEELNDIYQRVFPDE SIEFRTDNLEQIKEVLLDNKRSRADRQRTLVSDIYQSSEDKDIEKRNKAV ATEILKASLGNKAKLNVITNVEVDKEAAKEWSITFDSESIDDDLAKIEGQ MTDDGHEIIEVLRSLYSGITLSAIVPENHTLSQSMVAKYDLHKDHLKLFK KLINGMTDTKKAKNLRAAYDGYIDGVKGKVLPQEDFYKQVQVNLDDS AEANEIQTYIDQDIFMPKQRTKANGSIPHQLQQQELDQIIENQKAYYPWL AELNPNPDKKRQQLAKYKLDELVTFRVPYYVGPMITAKDQKNQSGAEF AWMIRKEPGNITPWNFDQKVDRMATANQFIKRMTTTDTYLLGEDVLPA QSLLYQKFEVLNELNKIRIDHKPISIEQKQQIFNDLFKQFKNVTIKHLQDY LVSQGQYSKRPLIEGLADEKRFNSSLSTYSDLCGIFGAKLVEENDRQEDL EKIIEWSTIFEDKKIYRAKLNDLTWLTDDQKEKLATKRYQGWGRLSRKL LVGLKNSEHRNIMDILWITNENFMQIQAEPDFAKLVTDANKGMLEKTDS QDVINDLYTSPQNKKAIRQILLVVHDIQNAMHGQAPAKIHVEFARGEER NPRRSVQRQRQVEAAYEKVSNELVSAKVRQEFKEAINNKRDFKDRLFL YFMQGGIDIYTGKQLNIDQLSSYQIDHILPQAFVKDDSLTNRVLTNENQV KADSVPIDIFGKKMLSVWGRMKDQGLISKGKYRNLTMNPENISAHTENG FINRQLVETRQVIKLAVNILADEYGDSTQIISVKADLSHQMREDFELLKN RDVNDYHHAFDAYLAAFIGNYLLKRYPKLESYFVYGDFKKFTQKETKM RRFNFIYDLKHCDQVVNKETGEILWTKDEDIKYIRHLFAYKKILVSHEVR EKRGALYNQTIYKAKDDKGSGQESKKLIRIKDDKETKIYGGYSGKSLAY MTIVQITKKNKVSYRVIGIPTLALARLNKLENDSTENNGELYKIIKPQFTH YKVDKKNGEIIETTDDFKIVVSKVRFQQLIDDAGQFFMLASDTYKNNAQ QLVISNNALKAINNTNITDCPRDDLERLDNLRLDSAFDEIVKKMDKYFSA YDANNFREKIRNSNLIFYQLPVEDQWENNKITELGKRTVLTRILQGLHAN ATTTDMSIFKIKTPFGQLRQRSGISLSENAQLIYQSPTGLFERRVQLNKIK (SEQ ID NO: 31)
FnCas9 *Fusobacterium nucleatum* NCBI Reference Sequence: WP_060798984.1	MKKQKFSDYYLGFDIGTNSVGWCVTDLDYNVLRFNKKDMWGSRLFEE AKTAAERRVQRNSRRRLKRRKWRLNLLEEIFSNEILKIDSNFFRRLKESSL WLEDKSSKEKFTLFNDDNYKDYDFYKQYPTIFHLRNELIKNPEKKDIRLV YLAIHSIFKSRGHFLFEGQNLKEIKNFETLYNNLIAFLEDNGINKIIDKNNI EKLEKIVCDSKKGLKDKEKEFKEIFNSDKQLVAIFKLSVGSSVSLNDLFD TDEYKKGEVEKEKISFREQIYEDDKPIYYSILGEKIELLDIAKTFYDFMVL NNILADSQYISEAKVKLYEEHKKDLKNLKYIIRKYNKGNYDKLFKDKNE NNYSAYIGLNKEKSKKEVIEKSRLKIDDLIKNIKGYLPKVEEIEEKDKAIF NKILNKIELKTILPKQRISDNGTLPYQIHEAELEKILENQSKYYDFLNYEE NGIITKDKLLMTFKFRIPYYVGPLNSYHKDKGGNSWIVRKEEGKILPWNF EQKVDIEKSAEEFIKRMTNKCTYLNGEDVIPKDTFLYSEYVILNELNKVQ VNDEFLNEENKRKIIDELFKENKKVSEKKFKEYLLVKQIVDGTIELKGVK DSFNSNYISYIRFKDIFGEKLNLDIYKEISEKSILWKCLYGDDKKIFEKKIK NEYGDILTKDEIKKINTFKFNNWGRLSEKLLTGIEFINLETGECYSSVMDA LRRTNYNLMELLSSKFTLQESINNENKEMNEASYRDLIEESYVSPSLKRAI FQTLKIYEEIRKITGRVPKKVFIEMARGGDESMKNKKIPARQEQLKKLYD SCGNDIANFSIDIKEMKNSLISYDNNSLRQKKLYLYYLQFGKCMYTGREI DLDRLLQNNDTYDIDHIYPRSKVIKDDSFDNLVLVLKNENAEKSNEYPV KKEIQEKMKSFWRFLKEKNFISDEKYKRLTGKDDFELRGFMARQLVNV RQTTKEVGKILQQIEPEIKIVYSKAEIASSFREMFDFIKVRELNDTHHAKD AYLNIVAGNVYNTKFTEKPYRYLQEIKENYDVKKIYNYDIKNAWDKEN SLEIVKKNMEKNTVNITRFIKEKKGQLFDLNPIKKGETSNEIISIKPKVYN

Description	Sequence
	GKDDKLNEKYGYYKSLNPAYFLYVEHKEKNKRIKSFERVNLVDVNNIK DEKSLVKYLIENKKLVEPRVIKKVYKRQVILINDYPYSIVTLDSNKLMDF ENLKPLFLENKYEKILKNVIKFLEDNQGKSEENYKFIYLKKKDRYEKNET LESVKDRYNLEFNEMYDKFLEKLDSKDYKNYMNNKKYQELLDVKEKFI KLNLFDKAFTLKSFLDLFNRKTMADFSKVGLTKYLGKIQKISSNVLSKNE LYLLEESVTGLFVKKIKL (SEQ ID NO: 32)
EcCas9 *Enterococcus cecorum* NCBI Reference Sequence: WP_047338501.1 Wild type	RRKQRIQILQELLGEEVLKTDPGFFHRMKESRYVVEDKRTLDGKQVELP YALFVDKDYTDKEYYKQFPTINHLIVYLMTTSDTPDIRLVYLALHYYMK NRGNFLHSGDINNVKDINDILEQLDNVLETFLDGWNLKLKSYVEDIKNIY NRDLGRGERKKAFVNTLGAKTKAEKAFCSLISGGSTNLAELFDDSSLKEI ETPKIEFASSSLEDKIDGIQEALEDRFAVIEAAKRLYDWKTLTDILGDSSS LAEARVNSYQMHHEQLLELKSLVKEYLDRKVFQEVFVSLNVANNYPAY IGHTKINGKKKELEVKRTKRNDFYSYVKKQVIEPIKKKVSDEAVLTKLSE IESLIEVDKYLPLQVNSDNGVIPYQVKLNELTRIFDNLENRIPVLRENRDK IIKTFKFRIPYYVGSLNGVVKNGKCTNWMVRKEEGKIYPWNFEDKVDLE ASAEQFIRRMTNKCTYLVNEDVLPKYSLLYSKYLVLSELNNLRIDGRPLD VKIKQDIYENVFKKNRKVTLKKIKKYLLKEGIITDDDELSGLADDVKSSL TAYRDFKEKLGHLDLSEAQMENIILNITLFGDDKKLLKKRLAALYPFIDD KSLNRIATLNYRDWGRLSERFLSGITSVDQETGELRTIIQCMYETQANLM QLLAEPYHFVEAIEKENPKVDLESISYRIVNDLYVSPAVKRQIWQTLLVIK DIKQVMKHDPERIFIEMAREKQESKKTKSRKQVLSEVYKKAKEYEHLFE KLNSLTEEQLRSKKIYLYFTQLGKCMYSGEPIDFENLVSANSNYDIDHIYP QSKTIDDSFNNIVLVKKSLNAYKSNHYPIDKNIRDNEKVKTLWNTLVSK GLITKEKYERLIRSTPFSDEELAGFIARQLVETRQSTKAVAEILSNWFPESE IVYSKAKNVSNFRQDFEILKVRELNDCHHAHDAYLNIVVGNAYHTKFTN SPYRFIKNKANQEYNLRKLLQKVNKIESNGVVAWVGQSENNPGTIATVK KVIRRNTVLISRMVKEVDGQLFDLTLMKKGKGQVPIKSSDERLTDISKY GGYNKATGAYFTFVKSKKRGKVVRSFEYVPLHLSKQFENNNELLKEYIE KDRGLTDVEILIPKVLINSLFRYNGSLVRITGRGDTRLLLVHEQPLYVSNS FVQQLKSVSSYKLKKSENDNAKLTKTATEKLSNIDELYDGLLRKLDLPIY SYWFSSIKEYLVESRTKYIKLSIEEKALVIFEILHLFQSDAQVPNLKILGLS TKPSRIRIQKNLKDTDKMSIIHQSPSGIFEHEIELTSL (SEQ ID NO: 33)
AhCas9 *Anaerostipes hadrus* NCBI Reference Sequence: WP_044924278.1 Wild type	MQNGFLGITVSSEQVGWAVTNPKYELERASRKDLWGVRLFDKAETAED RRMFRTNRRLNQRKKNRIHYLRDIFHEEVNQKDPNFFQQLDESNFCEDD RTVEFNFDTNLYKNQFPTVYHLRKYLMETKDKPDIRLVYLAFSKFMKN RGHFLYKGNLGEVMDFENSMKGFCESLEKFNIDFPTLSDEQVKEVRDIL CDHKIAKTVKKKNIITITKVKSKTAKAWIGLFCGCSVPVKVLFQDIDEEIV TDPEKISFEDASYDDYIANIEKGVGIYYEAIVSAKMLFDWSILNEILGDHQ LLSDAMIAEYNKHHDDLKRLQKIIKGTGSRELYQDIFINDVSGNYVCYV GHAKTMSSADQKQFYTFLKNRLKNVNGISSEDAEWIDTEIKNGTLLPKQ TKRDNSVIPHQLQLREFELILDNMQEMYPFLKENREKLLKIFNFVIPYYV GPLKGVVRKGESTNWMVPKKDGVIHPWNFDEMVDKEASAECFISRMT GNCSYLFNEKVLPKNSLLYETFEVLNELNPLKINGEPISVELKQRIYEQLF LTGKKVTKKSLTKYLIKNGYDKDIELSGIDNEFHSNLKSHIDFEDYDNLS DEEVEQIILRITVFEDKQLLKDYLNREFVKLSEDERKQICSLSYKGWGNL SEMLLNGITVTDSNGVEVSVMDMLWNTNLNLMQILSKKYGYKAEIEHY NKEHEKTIYNREDLMDYLNIPPAQRRKVNQLITIVKSLKKTYGVPNKIFF KISREHQDDPKRTSSRKEQLKYLYKSLKSEDEKHLMKELDELNDHELSN

Description	Sequence
	DKVYLYFLQKGRCIYSGKKLNLSRLRKSNYQNDIDYIYPLSAVNDRSMN NKVLTGIQENRADKYTYFPVDSEIQKKMKGFWMELVLQGFMTKEKYFR LSRENDFSKSELVSFIEREISDNQQSGRMIASVLQYYFPESKIVFVKEKLIS SFKRDFHLISSYGHNHLQAAKDAYITIVVGNVYHTKFTMDPAIYFKNHK RKDYDLNRLFLENISRDGQIAWESGPYGSIQTVRKEYAQNHIAVTKRVV EVKGGLFKQMPLKKGHGEYPLKTNDPRFGNIAQYGGYTNVTGSYFVLV ESMEKGKKRISLEYVPVYLHERLEDDPGHKLLKEYLVDHRKLNHPKILL AKVRKNSLLKIDGFYYRLNGRSGNALILTNAVELIMDDWQTKTANKISG YMKRRAIDKKARVYQNEFHIQELEQLYDFYLDKLKNGVYKNRKNNQA ELIHNEKEQFMELKTEDQCVLLTEIKKLFVCSPMQADLTLIGGSKHTGMI AMSSNVTKADFAVIAEDPLGLRNKVIYSHKGEK (SEQ ID NO: 34)
KvCas9 *Kandleria vitulina* NCBI Reference Sequence: WP_031589969.1 Wild type	MSQNNNKIYNIGLDIGDASVGWAVVDEHYNLLKRHGKHMWGSRLFTQ ANTAVERRSSRSTRRRYNKRRERIRLLREIMEDMVLDVDPTFFIRLANVS FLDQEDKKDYLKENYHSNYNLFIDKDFNDKTYYDKYPTIYHLRKHLCES KEKEDPRLIYLALHHIVKYRGNFLYEGQKFSMDVSNIEDKMIDVLRQFN EINLFEYVEDRKKIDEVLNVLKEPLSKKHKAEKAFALFDTTKDNKAAYK ELCAALAGNKFNVTKMLKEAELHDEDEKDISFKFSDATFDDAFVEKQPL LGDCVEFIDLLHDIYSWVELQNILGSAHTSEPSISAAMIQRYEDHKNDLK LLKDVIRKYLPKKYFEVFRDEKSKKNNYCNYINHPSKTPVDEFYKYIKK LIEKIDDPDVKTILNKIELESFMLKQNSRTNGAVPYQMQLDELNKILENQ SVYYSDLKDNEDKIRSILTFRIPYYFGPLNITKDRQFDWIIKKEGKENERIL PWNANEIVDVDKTADEFIKRMRNFCTYFPDEPVMAKNSLTVSKYEVLN EINKLRINDHLIKRDMKDKMLHTLFMDHKSISANAMKKWLVKNQYFSN TDDIKIEGFQKENACSTSLTPWIDFTKIFGKINESNYDFIEKIIYDVTVFED KKILRRRLKKEYDLDEEKIKKILKLKYSGWSRLSKKLLSGIKTKYKDSTR TPETVLEVMERTNMNLMQVINDEKLGFKKTIDDANSTSVSGKFSYAEVQ ELAGSPAIKRGIWQALLIVDEIKKIMKHEPAHVYIEFARNEDEKERKDSF VNQMLKLYKDYDFEDETEKEANKHLKGEDAKSKIRSERLKLYYTQMG KCMYTGKSLDIDRLDTYQVDHIVPQSLLKDDSIDNKVLVLSSENQRKLD DLVIPSSIRNKMYGFWEKLFNNKIISPKKFYSLIKTEFNEKDQERFINRQIV ETRQITKHVAQIIDNHYENTKVVTVRADLSHQFRERYHIYKNRDINDFHH AHDAYIATILGTYIGHRFESLDAKYIYGEYKRIFRNQKNKGKEMKKNND GFILNSMRNIYADKDTGEIVWDPNYIDRIKKCFYYKDCFVTKKLEENNG TFFNVTVLPNDTNSDKDNTLATVPVNKYRSNVNKYGGFSGVNSFIVAIK GKKKKGKKVIEVNKLTGIPLMYKNADEEIKINYLKQAEDLEEVQIGKEIL KNQLIEKDGGLYYIVAPTEIINAKQLILNESQTKLVCEIYKAMKYKNYDN LDSEKIIDLYRLLINKMELYYPEYRKQLVKKFEDRYEQLKVISIEEKCNII KQILATLHCNSSIGKIMYSDFKISTTIGRLNGRTISLDDISFIAESPTGMYSK KYKL (SEQ ID NO: 35)
EfCas9 *Enterococcus faecalis* NCBI Reference Sequence: WP_016631044.1	MRLFEEGHTAEDRRLKRTARRRISRRRNRLRYLQAFFEEAMTDLDENFF ARLQESFLVPEDKKWHRHPIFAKLEDEVAYHETYPTIYHLRKKLADSSE QADLRLIYLALAHIVKYRGHFLIEGKLSTENTSVKDQFQQFMVIYNQTFV NGESRLVSAPLPESVLIEEELTEKASRTKKSEKVLQQFPQEKANGLFGQF LKLMVGNKADFKKVFGLEEEAKITYASESYEEDLEGILAKVGDEYSDVF LAAKNVYDAVELSTILADSDKKSHAKLSSSMIVRFTEHQEDLKKFKRFIR ENCPDEYDNLFKNEQKDGYAGYIAHAGKVSQLKFYQYVKKIIQDIAGAE YFLEKIAQENFLRKQRTFDNGVIPHQIHLAELQAIIHRQAAYYPFLKENQE

Description	Sequence
Wild type	KIEQLVTFRIPYYVGPLSKGDASTFAWLKRQSEEPIRPWNLQETVDLDQS ATAFIERMTNFDTYLPSEKVLPKHSLLYEKFMVFNELTKISYTDDRGIKA NFSGKEKEKIFDYLFKTRRKVKKKDIIQFYRNEYNTEIVTLSGLEEDQFN ASFSTYQDLLKCGLTRAELDHPDNAEKLEDIIKILTIFEDRQRIRTQLSTFK GQFSAEVLKKLERKHYTGWGRLSKKLINGIYDKESGKTILDYLVKDDGV SKHYNRNFMQLINDSQLSFKNAIQKAQSSEHEETLSETVNELAGSPAIKK GIYQSLKIVDELVAIMGYAPKRIVVEMARENQTTSTGKRRSIQRLKIVEK AMAEIGSNLLKEQPTTNEQLRDTRLFLYYMQNGKDMYTGDELSLHRLS HYDIDHIIPQSFMKDDSLDNLVLVGSTENRGKSDDVPSKEVVKDMKAY WEKLYAAGLISQRKFQRLTKGEQGGLTLEDKAHFIQRQLVETRQITKNV AGILDQRYNAKSKEKKVQIITLKASLTSQFRSIFGLYKVREVNDYHHGQD AYLNCVVATTLLKVYPNLAPEFVYGEYPKFQTFKENKATAKAIIYTNLL RFFTEDEPRFTKDGEILWSNSYLKTIKKELNYHQMNIVKKVEVQKGGFS KESIKPKGPSNKLIPVKNGLDPQKYGGFDSPVVAYTVLFTHEKGKKPLIK QEILGITIMEKTRFEQNPILFLEEKGFLRPRVLMKLPKYTLYEFPEGRRRL LASAKEAQKGNQMVLPEHLLTLLYHAKQCLLPNQSESLAYVEQHQPEF QEILERVVDFAEVHTLAKSKVQQIVKLFEANQTADVKEIAASFIQLMQFN AMGAPSTFKFFQKDIERARYTSIKEIFDATIIYQSPTGLYETRRKVVD (SEQ ID NO: 36)
Staphylococcus aureus Cas9	KRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSKR GARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKL SEEEFSAALLHLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKY VAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFI DTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSV KYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTL KQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAEL LDQIAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAI NLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVK RSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTN ERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNY EVDHIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKISYETF KKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTRYATR GLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKHHA EDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYK EIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTLIV NNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDE KNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYP NSRNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKC YEEAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMI DITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQ IIKKG (SEQ ID NO: 37)
Geobacillus thermodenitrificans Cas9	MKYKIGLDIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRL ARSARRRLRRRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRV EALDRKLNNDELARILLHLAKRRGFRSNRKSERTNKENSTMLKHIEENQ SILSSYRTVAEMVVKDPKFSLHKRNKEDNYTNTVARDDLEREIKLIFAKQ REYGNIVCTEAFEHEYISIWASQRPFASKDDIEKKVGFCTFEPKEKRAPK ATYTFQSFTVWEHINKLRLVSPGGIRALTDDERRLIYKQAFHKNKITFHD

Description	Sequence
	VRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFLELGAYHKIRKAIDSVY GKGAAKSFRPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLA DKVYDEELIEELLNLSFSKFGHLSLKALRNILPYMEQGEVYSTACERAGY TFTGPKKKQKTVLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHI ELARELSQSFDERRKMQKEQEGNRKKNETAIRQLVEYGLTLNPTGLDIV KFKLWSEQNGKCAYSLQPIEIERLLEPGYTEVDHVIPYSRSLDDSYTNKV LVLTKENREKGNRTPAEYLGLGSERWQQFETFVLTNKQFSKKKRDRLLR LHYDENEENEFKNRNLNDTRYISRFLANFIREHLKFADSDDKQKVYTVN GRITAHLRSRWNFNKNREESNLHHAVDAAIVACTTPSDIARVTAFYQRR EQNKELSKKTDPQFPQPWPHFADELQARLSKNPKESIKALNLGNYDNEK LESLQPVFVSRMPKRSITGAAHQETLRRYIGIDERSGKIQTVVKKKLSEIQ LDKTGHFPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGE LGPIIRTIKIIDTTNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPIYTI DMMKGILPNKAIEPNKPYSEWKEMTEDYTFRFSLYPNDLIRIEFPREKTIK TAVGEEIKIKDLFAYYQTIDSSNGGLSLVSHDNNFSLRSIGSRTLKRFEKY QVDVLGNIYKVRGEKRVGVASSSHSKAGETIRPL (SEQ ID NO: 38)
ScCas9 *S. canis* 1375 AA 159.2 kDa	MEKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTNRKSIKKNLM GALLFDSGETAEATRLKRTARRRYTRRKNRIRYLQEIFANEMAKLDDSF FQRLEESFLVEEDKKNERHPIFGNLADEVAYHRNYPTIYHLRKKLADSPE KADLRLIYLALAHIIKFRGHFLIEGKLNAENSDVAKLFYQLIQTYNQLFEE SPLDEIEVDAKGILSARLSKSKRLEKLIAVFPNEKKNGLFGNIIALALGLTP NFKSNFDLTEDAKLQLSKDTYDDDLDELLGQIGDQYADLFSAAKNLSDA ILLSDILRSNSEVTKAPLSASMVKRYDEHHQDLALLKTLVRQQFPEKYAE IFKDDTKNGYAGYVDGIGIKHRKRTTKLATQEEFYKFIKPILEKMDGAEEL LAKLNRDDLLRKQRTFDNGSIPHQIHLKELHAILRRQEEFYPFLKENREKI EKILTFRIPYYVGPLARGNSRFAWLTRKSEEAITPWNFEEVVDKGASAQS FIERMTNFDEQLPNKKVLPKHSLLYEYFTVYNELTKVKYVTERMRKPEF LSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEIIGVEDRFNA SLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYA HLFDDKVMKQLKRRHYTGWGRLSRKMINGIRDKQSGKTILDFLKSDGF SNRNFMQLIHDDSLTFKEEIEKAQVSGQGDSLHEQIADLAGSPAIKKGIL QTVKIVDELVKVMGHKPENIVIEMARENQTTTKGLQQSRERKKRIEEGIK ELESQILKENPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDV DHIVPQSFIKDDSIDNKVLTRSVENRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSEADKAGFIKRQLVETRQITKHVARILDS RMNTKRDKNDKPIREVKVITLKSKLVSDFRKDFQLYKVRDINNYHHAH DAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKAT AKRFFYSNIMNFFKTEVKLANGEIRKRPLIETNGETGEVVWNKEKDFAT VRKVLAMPQVNIVKKTEVQTGGFSKESILSKRESAKLIPRKKGWDTRKY GGFGSPTVAYSILVVAKVEKGKAKKLKSVKVLVGITIMEKGSYEKDPIGF LEAKGYKDIKKELIFKLPKYSLFELENGRRRMLASATELQKANELVLPQ HLVRLLYYTQNISATTGSNNLGYIEQHREEFKEIFEKIIDFSEKYILKNKV NSNLKSSFDEQFAVSDSILLSNSFVSLLKYTSFGASGGFTFLDLDVKQGRL RYQTVTEVLDATLIYQSITGLYETRTDLSQLGGD (SEQ ID NO: 39)

[0313] The prime editors described herein may include any of the above Cas9 ortholog sequences, or any variants thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0314] The napDNAbp may include any suitable homologs and/or orthologs or naturally occurring enzymes, such as, Cas9. Cas9 homologs and/or orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Preferably, the Cas moiety is configured (e.g, mutagenized, recombinantly engineered, or otherwise obtained from nature) as a nickase, i.e., capable of cleaving only a single strand of the target doubpdditional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 nuclease has an inactive (e.g., an inactivated) DNA cleavage domain, that is, the Cas9 is a nickase.In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 3. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a Cas9 protein as provided by any one of the Cas9 orthologs in the above tables.

C. Dead Cas9 variant

[0315] In certain embodiments, the prime editors described herein may include a dead Cas9, e.g., dead SpCas9, which has no nuclease activity due to one or more mutations that inactive both nuclease domains of Cas9, namely the RuvC domain (which cleaves the non-protospacer DNA strand) and HNH domain (which cleaves the protospacer DNA strand). The nuclease inactivation may be due to one or mutations that result in one or more substitutions and/or deletions in the amino acid sequence of the encoded protein, or any variants thereof having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0316] As used herein, the term "dCas9" refers to a nuclease-inactive Cas9 or nuclease-dead Cas9, or a functional fragment thereof, and embraces any naturally occurring dCas9 from any organism, any naturally-occurring dCas9 equivalent or functional fragment thereof, any

dCas9 homolog, ortholog, or paralog from any organism, and any mutant or variant of a dCas9, naturally-occurring or engineered. The term dCas9 is not meant to be particularly limiting and may be referred to as a "dCas9 or equivalent." Exemplary dCas9 proteins and method for making dCas9 proteins are further described herein and/or are described in the art and are incorporated herein by reference.

[0317] In other embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity. In other embodiments, Cas9 variants having mutations other than D10A and H840A are provided which may result in the full or partial inactivate of the endogneous Cas9 nuclease acivity (e.g., nCas9 or dCas9, respectively). Such mutations, by way of example, include other amino acid substitutions at D10 and H820, or other substitutions within the nuclease domains of Cas9 (e.g., substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain) with reference to a wild type sequence such as Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1). In some embodiments, variants or homologues of Cas9 (e.g., variants of Cas9 from *Streptococcus pyogenes* (NCBI Reference Sequence: NC_017053.1 (SEQ ID NO: 20))) are provided which are at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to NCBI Reference Sequence: NC_017053.1. In some embodiments, variants of dCas9 (e.g., variants of NCBI Reference Sequence: NC_017053.1 (SEQ ID NO: 20)) are provided having amino acid sequences which are shorter, or longer than NC_017053.1 (SEQ ID NO: 20) by about 5 amino acids, by about 10 amino acids, by about 15 amino acids, by about 20 amino acids, by about 25 amino acids, by about 30 amino acids, by about 40 amino acids, by about 50 amino acids, by about 75 amino acids, by about 100 amino acids or more.

[0318] In one embodiment, the dead Cas9 may be based on the canonical SpCas9 sequence of Q99ZW2 and may have the following sequence, which comprises a D10X and an H810X, wherein X may be any amino acid, substitutions (underlined and bolded), or a variant be variant of SEQ ID NO: 40 having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

[0319] In one embodiment, the dead Cas9 may be based on the canonical SpCas9 sequence of Q99ZW2 and may have the following sequence, which comprises a D10A and an H810A

substitutions (underlined and bolded), or be a variant of SEQ ID NO: 41 having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

Description	Sequence	SEQ ID NO:
dead Cas9 or dCas9 *Streptococcus pyogenes* Q99ZW2 Cas9 with D10**X** and H810**X** Where "X" is any amino acid	MDKKYSIGL**X**IGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVD**X**IVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 40
dead Cas9 or dCas9 *Streptococcus pyogenes* Q99ZW2 Cas9 with D10**A** and H810**A**	MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN	SEQ ID NO: 41

Description	Sequence	SEQ ID NO:
	GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVD**A**IVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	

D. Cas9 nickase variant

[0320] In one embodment, the prime editors described herein comprise a Cas9 nickase. The term "Cas9 nickase" of "nCas9" refers to a variant of Cas9 which is capable of introducing a single-strand break in a double strand DNA molecule target. In some embodiments, the Cas9 nickase comprises only a single functioning nuclease domain. The wild type Cas9 (e.g., the canonical SpCas9) comprises two separate nuclease domains, namely, the RuvC domain (which cleaves the non-protospacer DNA strand) and HNH domain (which cleaves the protospacer DNA strand). In one embodiment, the Cas9 nickase comprises a mutation in the RuvC domain which inactivates the RuvC nuclease activity. For example, mutations in aspartate (D) 10, histidine (H) 983, aspartate (D) 986, or glutamate (E) 762, have been reported as loss-of-function mutations of the RuvC nuclease domain and the creation of a functional Cas9 nickase (e.g., Nishimasu et al., "Crystal structure of Cas9 in complex with guide RNA and target DNA," *Cell* 156(5), 935–949, which is incorporated herein by reference). Thus, nickase mutations in the RuvC domain could include D10X, H983X, D986X, or E762X, wherein X is any amino acid other than the wild type amino acid. In

certain embodiments, the nickase could be D10A, of H983A, or D986A, or E762A, or a combination thereof.

[0321] In various embodiments, the Cas9 nickase can having a mutation in the RuvC nuclease domain and have one of the following amino acid sequences, or a variant thereof having an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

Description	Sequence	SEQ ID NO:
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with D10**X**, wherein X is any alternate amino acid	MDKKYSIGL**X**IGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 42
Cas9 nickase *Streptococcus pyogenes*	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL	SEQ ID NO: 43

Description	Sequence	SEQ ID NO:
Q99ZW2 Cas9 with E762X, wherein X is any alternate amino acid	AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIXMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with H983X, wherein X is any alternate amino acid	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD	SEQ ID NO: 44

Description	Sequence	SEQ ID NO:
	KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYH**X** AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with D986X, wherein X is any alternate amino acid	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AH**X**AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI	SEQ ID NO: 45

Description	Sequence	SEQ ID NO:
	DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with D10**A**	MDKKYSIGL**A**IGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 46
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with E762A	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS	SEQ ID NO: 47

Description	Sequence	SEQ ID NO:
	ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with H983A	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL	SEQ ID NO: 48

Description	Sequence	SEQ ID NO:
	DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYH**A** AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with D986A	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AH**A**AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH	SEQ ID NO: 49

Description	Sequence	SEQ ID NO:
	RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	

[0322] In another embodiment, the Cas9 nickase comprises a mutation in the HNH domain which inactivates the HNH nuclease activity. For example, mutations in histidine (H) 840 or asparagine (R) 863 have been reported as loss-of-function mutations of the HNH nuclease domain and the creation of a functional Cas9 nickase (e.g., Nishimasu et al., "Crystal structure of Cas9 in complex with guide RNA and target DNA," *Cell* 156(5), 935–949, which is incorporated herein by reference). Thus, nickase mutations in the HNH domain could include H840X and R863X, wherein X is any amino acid other than the wild type amino acid. In certain embodiments, the nickase could be H840A or R863A or a combination thereof.

[0323] In various embodiments, the Cas9 nickase can have a mutation in the HNH nuclease domain and have one of the following amino acid sequences, or a variant thereof having an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

Description	Sequence	SEQ ID NO:
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with H840**X**, wherein X is any alternate amino acid	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVD**X**IVPQSFLKDDSIDNKVLTRSDKNRGKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP	SEQ ID NO: 50

Description	Sequence	SEQ ID NO:
	LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with H840**A**	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVD**A**IVPQSFLKDDSIDNKVLTRSDKN**R**GKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 51
Cas9 nickase *Streptococcus pyogenes* Q99ZW2	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE	SEQ ID NO: 52

Description	Sequence	SEQ ID NO:
Cas9 with R863X, wherein X is any alternate amino acid	NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKN**X**GKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	
Cas9 nickase *Streptococcus pyogenes* Q99ZW2 Cas9 with R863**A**	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTD RHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF GNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEE NPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRE DLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDN REKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRD KQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ	SEQ ID NO: 53

Description	Sequence	SEQ ID NO:
	VSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQEL DINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKN**A**GKS DNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAER GGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRK MIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRP LIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTS TKEVLDATLIHQSITGLYETRIDLSQLGGD	

[0324] In some embodiments, the N-terminal methionine is removed from a Cas9 nickase, or from any Cas9 variant, ortholog, or equivalent disclosed or contemplated herein. For example, methionine-minus Cas9 nickases include the following sequences, or a variant thereof having an amino acid sequence that has at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity thereto.

Description	Sequence
Cas9 nickase (Met minus) *Streptococcus pyogenes* Q99ZW2 Cas9 with H840**X**, wherein X is any alternate amino acid	DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIG ALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSF FHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLA AKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREK IEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASA QSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILD FLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV DQELDINRLSDYDVD**X**IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKR QLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFR KDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE

	TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILP KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITG LYETRIDLSQLGGD (SEQ ID NO: 54)
Cas9 nickase (Met minus) *Streptococcus pyogenes* Q99ZW2 Cas9 with H840**A**	DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIG ALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSF FHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLA AKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREK IEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASA QSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILD FLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV DQELDINRLSDYDVD**A**IVPQSFLKDDSIDNKVLTRSDKN**R**GKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKR QLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFR KDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILP KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITG LYETRIDLSQLGGD (SEQ ID NO: 55)
Cas9 nickase (Met minus) *Streptococcus pyogenes* Q99ZW2 Cas9 with R863X, wherein X is any alternate amino acid	DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIG ALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSF FHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLA AKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREK IEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASA QSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE

	RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILD FLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV DQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKN**X**GKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKR QLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFR KDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILP KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITG LYETRIDLSQLGGD(SEQ ID NO: 56)
Cas9 nickase (Met minus) *Streptococcus pyogenes* Q99ZW2 Cas9 with R863**A**	DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIG ALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSF FHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQ LFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLA AKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELL VKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREK IEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASA QSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMR KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILD FLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV DQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKN**A**GKSDNVPS EEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKR QLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFR KDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYK VYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILP KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLK SVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELEN GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIRE QAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITG LYETRIDLSQLGGD (SEQ ID NO: 57)

E. Other Cas9 variants

[0325] Besides dead Cas9 and Cas9 nickase variants, the Cas9 proteins used herein may also include other "Cas9 variants" having at least about 70% identical, at least about 80%

identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference Cas9 protein, including any wild type Cas9, or mutant Cas9 (e.g., a dead Cas9 or Cas9 nickase), or fragment Cas9, or circular permutant Cas9, or other variant of Cas9 disclosed herein or known in the art. In some embodiments, a Cas9 variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to a reference Cas9. In some embodiments, the Cas9 variant comprises a fragment of a reference Cas9 (*e.g.*, a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type Cas9. In some embodiments, the fragment is is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type Cas9 (*e.g.*, SEQ ID NO: 18).

[0326] In some embodiments, the disclosure also may utilize Cas9 fragments which retain their functionality and which are fragments of any herein disclosed Cas9 protein. In some embodiments, the Cas9 fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, or at least 1300 amino acids in length.

[0327] In various embodiments, the prime editors disclosed herein may comprise one of the Cas9 variants described as follows, or a Cas9 variant thereof having at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference Cas9 variants.

F. Small-sized Cas9 variants

[0328] In some embodiments, the prime editors contemplated herein can include a Cas9 protein that is of smaller molecular weight than the canonical SpCas9 sequence. In some embodiments, the smaller-sized Cas9 variants may facilitate delivery to cells, e.g., by an expression vector, nanoparticle, or other means of delivery. In certain embodiments, the smaller-sized Cas9 variants can include enzymes categorized as type II enzymes of the Class 2 CRISPR-Cas systems. In some embodiments, the smaller-sized Cas9 variants can include enzymes categorized as type V enzymes of the Class 2 CRISPR-Cas systems. In other embodiments, the smaller-sized Cas9 variants can include enzymes categorized as type VI enzymes of the Class 2 CRISPR-Cas systems.

[0329] The canonical SpCas9 protein is 1368 amino acids in length and has a predicted molecular weight of 158 kilodaltons. The term "small-sized Cas9 variant", as used herein, refers to any Cas9 variant—naturally occurring, engineered, or otherwise—that is less than at least 1300 amino acids, or at least less than 1290 amino acids, or than less than 1280 amino acids, or less than 1270 amino acid, or less than 1260 amino acid, or less than 1250 amino acids, or less than 1240 amino acids, or less than 1230 amino acids, or less than 1220 amino acids, or less than 1210 amino acids, or less than 1200 amino acids, or less than 1190 amino acids, or less than 1180 amino acids, or less than 1170 amino acids, or less than 1160 amino acids, or less than 1150 amino acids, or less than 1140 amino acids, or less than 1130 amino acids, or less than 1120 amino acids, or less than 1110 amino acids, or less than 1100 amino acids, or less than 1050 amino acids, or less than 1000 amino acids, or less than 950 amino acids, or less than 900 amino acids, or less than 850 amino acids, or less than 800 amino acids, or less than 750 amino acids, or less than 700 amino acids, or less than 650 amino acids, or less than 600 amino acids, or less than 550 amino acids, or less than 500 amino acids, but at least larger than about 400 amino acids and retaining the required functions of the Cas9 protein. The Cas9 variants can include those categorized as type II, type V, or type VI enzymes of the Class 2 CRISPR-Cas system.

[0330] In various embodiments, the prime editors disclosed herein may comprise one of the small-sized Cas9 variants described as follows, or a Cas9 variant thereof having at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference small-sized Cas9 protein.

Description	Sequence	SEQ ID NO:
SaCas9 *Staphylococcus aureus* 1053 AA 123 kDa	MGKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEA NVENNEGRRSKRGARRLKRRRRHRIQRVKKLLFDYNLL TDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLAKRRG VHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLE RLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLD QSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEML MGHCTYFPEELRSVKYAYNADLYNALNDLNNLVITRDE NEKLEYYEKFQIIENVFKQKKKPTLKQIAKEILVNEEDIK GYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIA KILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHN LSLKAINLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQK EIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYGLPNDIIIEL AREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENA KYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDH IIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDS KISYETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSV QKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSI NGGFTSFLRRKWKFKKERNKGYKHHAEDALIIANADFIF KEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEYKEI FITPHQIKHIKDFKDYKYSHRVDKKPNRKLINDTLYSTRK DDKGNTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYH HDPQTYQKLKLIMEQYGDEKNPLYKYYEETGNYLTKYS KKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVKLS LKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKC YEEAKKLKKISNQAEFIASFYKNDLIKINGELYRVIGVNN DLLNRIEVNMIDITYREYLENMNDKRPPHIIKTIASKTQSI KKYSTDILGNLYEVKSKKHPQIIKK	SEQ ID NO: 58
NmeCas9 *N. meningitidis* 1083 AA 124.5 kDa	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDL GVRVFERAEVPKTGDSLAMARRLARSVRRLTRRRAHRL LRTRRLLKREGVLQAANFDENGLIKSLPNTPWQLRAAAL DRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELGA LLKGVAGNAHALQTGDFRTPAELALNKFEKESGHIRNQR SDYSHTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIE TLLMTQRPALSGDAVQKMLGHCTFEPAEPKAAKNTYTA ERFIWLTKLNNLRILEQGSERPLTDTERATLMDEPYRKSK LTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKA YHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTD EDITGRLKDRIQPEILEALLKHISFDKFVQISLKALRRIVPL MEQGKRYDEACAEIYGDHYGKKNTEEKIYLPPIPADEIR NPVVLRALSQARKVINGVVRRYGSPARIHIETAREVGKSF KDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKSKD ILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDAALPF SRTWDDSFNNKVLVLGSENQNKGNQTPYEYFNGKDNSR EWQEFKARVETSRFPRSKKQRILLQKFDEDGFKERNLND TRYVNRFLCQFVADRMRLTGKGKKRVFASNGQITNLLR GFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRF VRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQE VMIRVFGKPDGKPEFEEADTLEKLRTLLAEKLSSRPEAVH	SEQ ID NO: 59

Description	Sequence	SEQ ID NO:
	EYVTPLFVSRAPNRKMSGQGHMETVKSAKRLDEGVSVL RVPLTQLKLKDLEKMVNREREPKLYEALKARLEAHKDD PAKAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWV RNHNGIADNATMVRVDVFEKGDKYYLVPIYSWQVAKGI LPDRAVVQGKDEEDWQLIDDSFNFKFSLHPNDLVEVITK KARMFGYFASCHRGTGNINIRIHDLDHKIGKNGILEGIGV KTALSFQKYQIDELGKEIRPCRLKKRPPVR	
CjCas9 *C. jejuni* 984 AA 114.9 kDa	MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKT GESLALPRRLARSARKRLARRKARLNHLKHLIANEFKLN YEDYQSFDESLAKAYKGSLISPYELRFRALNELLSKQDFA RVILHIAKRRGYDDIKNSDDKEKGAILKAIKQNEEKLAN YQSVGEYLYKEYFQKFKENSKEFTNVRNKKESYERCIAQ SFLKDELKLIFKKQREFGFSFSKKFEEEVLSVAFYKRALK DFSHLVGNCSFFTDEKRAPKNSPLAFMFVALTRIINLLNN LKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLS DDYEFKGEKGTYFIEFKKYKEFIKALGEHNLSQDDLNEIA KDITLIKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNIS FKALKLVTPLMLEGKKYDEACNELNLKVAINEDKKDFLP AFNETYYKDEVTNPVVLRAIKEYRKVLNALLKKYGKVH KINIELAREVGKNHSQRAKIEKEQNENYKAKKDAELECE KLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISDLQDEK MLEIDHIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFE AFGNDSAKWQKIEVLAKNLPTKKQKRILDKNYKDKEQK NFKDRNLNDTRYIARLVLNYTKDYLDFLPLSDDENTKLN DTQKGSKVHVEAKSGMLTSALRHTWGFSAKDRNNHLH HAIDAVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISEL DYKNKRKFFEPFSGFRQKVLDKIDEIFVSKPERKKPSGAL HEETFRKEEEFYQSYGGKEGVLKALELGKIRKVNGKIVK NGDMFRVDIFKHKKTNKFYAVPIYTMDFALKVLPNKAV ARSKKGEIKDWILMDENYEFCFSLYKDSLILIQTKDMQEP EFVYYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANE KEVIAKSIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK	SEQ ID NO: 60
GeoCas9 *G. stearothermophilus* 1087 AA 127 kDa	MRYKIGLDIGITSVGWAVMNLDIPRIEDLGVRIFDRAENP QTGESLALPRRLARSARRRLRRRKHRLERIRRLVIREGILT KEELDKLFEEKHEIDVWQLRVEALDRKLNNDELARVLL HLAKRRGFKSNRKSERSNKENSTMLKHIEENRAILSSYRT VGEMIVKDPKFALHKRNKGENYTNTIARDDLEREIRLIFS KQREFGNMSCTEEFENEYITIWASQRPVASKDDIEKKVGF CTFEPKEKRAPKATYTFQSFIAWEHINKLRLISPSGARGLT DEERRLLYEQAFQKNKITYHDIRTLLHLPDDTYFKGIVYD RGESRKQNENIRFLELDAYHQIRKAVDKVYGKGKSSSFL PIDFDTFGYALTLFKDDADIHSYLRNEYEQNGKRMPNLA NKVYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEV YSSACERAGYTFTGPKKKQKTMLLPNIPPIANPVVMRAL TQARKVVNAIIKKYGSPVSIHIELARDLSQTFDERRKTKK EQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQ NGRCAYSLQPIEIERLLEPGYVEVDHVIPYSRSLDDSYTN KVLVLTRENREKGNRIPAEYLGVGTERWQQFETFVLTNK	SEQ ID NO: 61

Description	Sequence	SEQ ID NO:
	QFSKKKRDRLLRLHYDENEETEFKNRNLNDTRYISRFFA NFIREHLKFAESDDKQKVYTVNGRVTAHLRSRWEFNKN REESDLHHAVDAVIVACTTPSDIAKVTAFYQRREQNKEL AKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGNY DDQKLESLQPVFVSRMPKRSVTGAAHQETLRRYVGIDER SGKIQTVVKTKLSEIKLDASGHFPMYGKESDPRTYEAIRQ RLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVKIIDTKN QVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPVYTMD IMKGILPNKAIEPNKPYSEWKEMTEDYTFRFSLYPNDLIRI ELPREKTVKTAAGEEINVKDVFVYYKTIDSANGGLELISH DHRFSLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVG LASSAHSKPGKTIRPLQSTRD	
LbaCas12a *L. bacterium* 1228 AA 143.9 kDa	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVED EKRAEDYKGVKKLLDRYYLSFINDVLHSIKLKNLNNYIS LFRKKTRTEKENKELENLEINLRKEIAKAFKGNEGYKSLF KKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNREN MFSEEAKSTSIAFRCINENLTRYISNMDIFEKVDAIFDKHE VQEIKEKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNAIIG GFVTESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVL SDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLE KLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRDKW NAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQ EYADADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFV LEKSLKKNDAVVAIMKDLLDSVKSFENYIKAFFGEGKET NRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKD KFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAI MDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPK VFFSKKWMAYYNPSEDIQKIYKNGTFKKGDMFNLNDCH KLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREV EEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKDFSDK SHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASL KKEELVVHPANSPIANKNPDNPKKTTTLSYDVYKDKRFS EDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGI DRGERNLLYIVVVDGKGNIVEQYSLNEIINNFNGIRIKTD YHSLLDKKEKERFEARQNWTSIENIKELKAGYISQVVHKI CELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKM LIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMST QNGFIFYIPAWLTSKIDPSTGFVNLLKTKYTSIADSKKFISS FDRIMYVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYG NRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGINYQQ GDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDV DFLISPVKNSDGIFYDSRNYEAQENAILPKNADANGAYNI ARKVLWAIGQFKKAEDEKLDKVKIAISNKEWLEYAQTS VKH	SEQ ID NO: 62
BhCas12b *B. hisashii*	MATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILK LIRQEAIYEHHEQDPKNPKKVSKAEIQAELWDFVLKMQK CNSFTHEVDKDEVFNILRELYEELVPSSVEKKGEANQLSN KFLYPLVDPNSQSGKGTASSGRKPRWYNLKIAGDPSWEE	SEQ ID NO: 63

Description	Sequence	SEQ ID NO:
1108 AA 130.4 kDa	EKKKWEEDKKKDPLAKILGKLAEYGLIPLFIPYTDSNEPI VKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNL KVKEEYEKVEKEYKTLEERIKEDIQALKALEQYEKERQE QLLRDTLNTNEYRLSKRGLRGWREIIQKWLKMDENEPSE KYLEVFKDYQRKHPREAGDYSVYEFLSKKENHFIWRNH PEYPYLYATFCEIDKKKKDAKQQATFTLADPINHPLWVR FEERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTE SGGWEEKGKVDIVLLPSRQFYNQIFLDIEEKGKHAFTYK DESIKFPLKGTLGGARVQFDRDHLRRYPHKVESGNVGRI YFNMTVNIEPTESPVSKSLKIHRDDFPKVVNFKPKELTEW IKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEV VDQKPDIEGKLFFPIKGTELYAVHRASFNIKLPGETLVKS REVLRKAREDNLKLMNQKLNFLRNVLHFQQFEDITEREK RVTKWISRQENSDVPLVYQDELIQIRELMYKPYKDWVAF LKQLHKRLEVEIGKEVKHWRKSLSDGRKGLYGISLKNID EIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNHLN ALKEDRLKKMANTIIMHALGYCYDVRKKKWQAKNPAC QIILFEDLSNYNPYEERSRFENSKLMKWSRREIPRQVALQ GEIYGLQVGEVGAQFSSRFHAKTGSPGIRCSVVTKEKLQ DNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGGEKFISLS KDRKCVTTHADINAAQNLQKRFWTRTHGFYKVYCKAY QVDGQTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVN AGKLKIKKGSSKQSSSELVDSDILKDSFDLASELKGEKLM LYRDPSGNVFPSDKWMAAGVFFGKLERILISKLTNQYSIS TIEDDSSKQSM	

G. Cas9 equivalents

[0331] In some embodiments, the prime editors described herein can include any Cas9 equivalent. As used herein, the term "Cas9 equivalent" is a broad term that encompasses any napDNAbp protein that serves the same function as Cas9 in the present prime editors despite that its amino acid primary sequence and/or its three-dimensional structure may be different and/or unrelated from an evolutionary standpoint. Thus, while Cas9 equivalents include any Cas9 ortholog, homolog, mutant, or variant described or embraced herein that are evolutionarily related, the Cas9 equivalents also embrace proteins that may have evolved through convergent evolution processes to have the same or similar function as Cas9, but which do not necessarily have any similarity with regard to amino acid sequence and/or three dimensional structure. The prime editors described here embrace any Cas9 equivalent that would provide the same or similar function as Cas9 despite that the Cas9 equivalent may be based on a protein that arose through convergent evolution. For instance, if Cas9 refers to a type II enzyme of the CRISPR-Cas system, a Cas9 equivalent can refer to a type V or type VI enzyme of the CRISPR-Cas system.

[0332] For example, Cas12e (CasX) is a Cas9 equivalent that reportedly has the same function as Cas9 but which evolved through convergent evolution. Thus, the Cas12e (CasX) protein described in Liu et al., "CasX enzymes comprises a distinct family of RNA-guided genome editors," *Nature*, 2019, Vol.566: 218-223, is contemplated to be used with the prime editors described herein. In addition, any variant or modification of Cas12e (CasX) is conceivable and within the scope of the present disclosure.

[0333] Cas9 is a bacterial enzyme that evolved in a wide variety of species. However, the Cas9 equivalents contemplated herein may also be obtained from archaea, which constitute a domain and kingdom of single-celled prokaryotic microbes different from bacteria.

[0334] In some embodiments, Cas9 equivalents may refer to Cas12e (CasX) or Cas12d (CasY), which have been described in, for example, Burstein et al., "New CRISPR–Cas systems from uncultivated microbes." *Cell Res.* 2017 Feb 21. doi: 10.1038/cr.2017.21, the entire contents of which is hereby incorporated by reference. Using genome-resolved metagenomics, a number of CRISPR–Cas systems were identified, including the first reported Cas9 in the archaeal domain of life. This divergent Cas9 protein was found in little-studied nanoarchaea as part of an active CRISPR–Cas system. In bacteria, two previously unknown systems were discovered, CRISPR– Cas12e and CRISPR– Cas12d, which are among the most compact systems yet discovered. In some embodiments, Cas9 refers to Cas12e, or a variant of Cas12e. In some embodiments, Cas9 refers to a Cas12d, or a variant of Cas12d. It should be appreciated that other RNA-guided DNA binding proteins may be used as a nucleic acid programmable DNA binding protein (napDNAbp), and are within the scope of this disclosure. Also see Liu et al., "CasX enzymes comprises a distinct family of RNA-guided genome editors," *Nature*, 2019, Vol.566: 218-223. Any of these Cas9 equivalents are contemplated.

[0335] In some embodiments, the Cas9 equivalent comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas12e (CasX) or Cas12d (CasY) protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12e (CasX) or Cas12d (CasY) protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a wild-type Cas moiety or any Cas moiety provided herein.

[0336] In various embodiments, the nucleic acid programmable DNA binding proteins include, without limitation, Cas9 (*e.g.,* dCas9 and nCas9), Cas12e (CasX), Cas12d (CasY), Cas12a (Cpf1), Cas12b1 (C2c1), Cas13a (C2c2), Cas12c (C2c3), Argonaute, , and Cas12b1. One example of a nucleic acid programmable DNA-binding protein that has different PAM specificity than Cas9 is Clustered Regularly Interspaced Short Palindromic Repeats from Prevotella and Francisella 1 (i.e, Cas12a (Cpf1)). Similar to Cas9, Cas12a (Cpf1) is also a Class 2 CRISPR effector, but it is a member of type V subgroup of enzymes, rather than the type II subgroup. It has been shown that Cas12a (Cpf1) mediates robust DNA interference with features distinct from Cas9. Cas12a (Cpf1) is a single RNA-guided endonuclease lacking tracrRNA, and it utilizes a T-rich protospacer-adjacent motif (TTN, TTTN, or YTN). Moreover, Cpf1 cleaves DNA via a staggered DNA double-stranded break. Out of 16 Cpf1-family proteins, two enzymes from *Acidaminococcus* and *Lachnospiraceae* are shown to have efficient genome-editing activity in human cells. Cpf1 proteins are known in the art and have been described previously, for example Yamano *et al.*, "Crystal structure of Cpf1 in complex with guide RNA and target DNA." *Cell* (165) 2016, p. 949-962; the entire contents of which is hereby incorporated by reference.

[0337] In still other embodiments, the Cas protein may include any CRISPR associated protein, including but not limited to, Cas12a, Cas12b1, Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof, and preferably comprising a nickase mutation (e.g., a mutation corresponding to the D10A mutation of the wild type Cas9 polypeptide of SEQ ID NO: 18).

[0338] In various other embodiments, the napDNAbp can be any of the following proteins: a Cas9, a Cas12a (Cpf1), a Cas12e (CasX), a Cas12d (CasY), a Cas12b1 (C2c1), a Cas13a (C2c2), a Cas12c (C2c3), a GeoCas9, a CjCas9, a Cas12g, a Cas12h, a Cas12i, a Cas13b, a Cas13c, a Cas13d, a Cas14, a Csn2, an xCas9, an SpCas9-NG, a circularly permuted Cas9, or an Argonaute (Ago) domain, or a variant thereof.

[0339] Exemplary Cas9 equivalent protein sequences can include the following:

Description	Sequence
AsCas12a (previously	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKEL KPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQA TYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTV

known as Cpf1) *Acidaminococcus sp. (strain BV3L6)* UniProtKB U2UMQ6	TTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPK FKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQ TQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIP LFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNE LNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKE KVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTL KKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEM EPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAI LFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAK MIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKF QTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKG HHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHR LGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVI TKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHP ETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERV AARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQ FTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFL EGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQF DAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSN ILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLNGV CFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISN QDWLAYIQELRN (SEQ ID NO: 64)
AsCas12a nickase (e.g., R1226A)	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKEL KPIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQA TYRNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTV TTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPK FKENCHIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQ TQIDLYNQLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIP LFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNE LNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKE KVQRSLKHEDINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTL KKQEEKEILKSQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEM EPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAI LFVKNGLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAK MIPKCSTQLKAVTAHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKF QTAYAKKTGDQKGYREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQY KDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKG HHGKPNLHTLYWTGLFSPENLAKTSIKLNGQAELFYRPKSRMKRMAHR LGEKMLNKKLKDQKTPIPDTLYQELYDYVNHRLSHDLSDEARALLPNVI TKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYLKEHP ETPIIGIDRGERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERV AARQAWSVVGTIKDLKQGYLSQVIHEIVDLMIHYQAVVVLENLNFGFK SKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAEKVGGVLNPYQLTDQ FTSFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFL EGFDFLHYDVKTGDFILHFKMNRNLSFQRGLPGFMPAWDIVFEKNETQF DAKGTPFIAGKRIVPVIENHRFTGRYRDLYPANELIALLEEKGIVFRDGSN ILPKLLENDDSHAIDTMVALIRSVLQMANSNAATGEDYINSPVRDLNGV

	CFDSRFQNPEWPMDADANGAYHIALKGQLLLNHLKESKDLKLQNGISN QDWLAYIQELRN (SEQ ID NO: 65)
LbCas12a (previously known as Cpf1) *Lachnospiraceae bacterium GAM79* Ref Seq. WP_119623382.1	MNYKTGLEDFIGKESLSKTLRNALIPTESTKIHMEEMGVIRDDELRAEKQ QELKEIMDDYYRTFIEEKLGQIQGIQWNSLFQKMEETMEDISVRKDLDKI QNEKRKEICCYFTSDKRFKDLFNAKLITDILPNFIKDNKEYTEEEKAEKE QTRVLFQRFATAFTNYFNQRRNNFSEDNISTAISFRIVNENSEIHLQNMR AFQRIEQQYPEEVCGMEEEYKDMLQEWQMKHIYSVDFYDRELTQPGIE YYNGICGKINEHMNQFCQKNRINKNDFRMKKLHKQILCKKSSYYEIPFR FESDQEVYDALNEFIKTMKKKEIIRRCVHLGQECDDYDLGKIYISSNKYE QISNALYGSWDTIRKCIKEEYMDALPGKGEKKEEKAEAAAKKEEYRSIA DIDKIISLYGSEMDRTISAKKCITEICDMAGQISIDPLVCNSDIKLLQNKEK TTEIKTILDSFLHVYQWGQTFIVSDIIEKDSYFYSELEDVLEDFEGITTLYN HVRSYVTQKPYSTVKFKLHFGSPTLANGWSQSKEYDNNAILLMRDQKF YLGIFNVRNKPDKQIIKGHEKEEKGDYKKMIYNLLPGPSKMLPKVFITSR SGQETYKPSKHILDGYNEKRHIKSSPKFDLGYCWDLIDYYKECIHKHPD WKNYDFHFSDTKDYEDISGFYREVEMQGYQIKWTYISADEIQKLDEKG QIFLFQIYNKDFSVHSTGKDNLHTMYLKNLFSEENLKDIVLKLNGEAELF FRKASIKTPIVHKKGSVLVNRSYTQTVGNKEIRVSIPEEYYTEIYNYLNHI GKGKLSSEAQRYLDEGKIKSFTATKDIVKNYRYCCDHYFLHLPITINFKA KSDVAVNERTLAYIAKKEDIHIIGIDRGERNLLYISVVDVHGNIREQRSFN IVNGYDYQQKLKDREKSRDAARKNWEEIEKIKELKEGYLSMVIHYIAQL VVKYNAVVAMEDLNYGFKTGRFKVERQVYQKFETMLIEKLHYLVFKD REVCEEGGVLRGYQLTYIPESLKKVGKQCGFIFYVPAGYTSKIDPTTGFV NLFSFKNLTNRESRQDFVGKFDEIRYDRDKKMFEFSFDYNNYIKKGTILA STKWKVYTNGTRLKRIVVNGKYTSQSMEVELTDAMEKMLQRAGIEYH DGKDLKGQIVEKGIEAEIIDIFRLTVQMRNSRSESEDREYDRLISPVLNDK GEFFDTATADKTLPQDADANGAYCIALKGLYEVKQIKENWKENEQFPR NKLVQDNKTWFDFMQKKRYL (SEQ ID NO: 66)
PcCas12a – previously known at Cpf1 *Prevotella copri* Ref Seq. WP_119227726.1	MAKNFEDFKRLYSLSKTLRFEAKPIGATLDNIVKSGLLDEDEHRAASYV KVKKLIDEYHKVFIDRVLDDGCLPLENKGNNNSLAEYYESYVSRAQDE DAKKKFKEIQQNLRSVIAKKLTEDKAYANLFGNKLIESYKDKEDKKKII DSDLIQFINTAESTQLDSMSQDEAKELVKEFWGFVTYFYGFFDNRKNMY TAEEKSTGIAYRLVNENLPKFIDNIEAFNRAITRPEIQENMGVLYSDFSEY LNVESIQEMFQLDYYNMLLTQKQIDVYNAIIGGKTDDEHDVKIKGINEYI NLYNQQHKDDKLPKLKALFKQILSDRNAISWLPEEFNSDQEVLNAIKDC YERLAENVLGDKVLKSLLGSLADYSLDGIFIRNDLQLTDISQKMFGNWG VIQNAIMQNIKRVAPARKHKESEEDYEKRIAGIFKKADSFSISYINDCLNE ADPNNAYFVENYFATFGAVNTPTMQRENLFALVQNAYTEVAALLHSDY PTVKHLAQDKANVSKIKALLDAIKSLQHFVKPLLGKGDESDKDERFYGE LASLWAELDTVTPLYNMIRNYMTRKPYSQKKIKLNFENPQLLGGWDAN KEKDYATIILRRNGLYYLAIMDKDSRKLLGKAMPSDGECYEKMVYKFF KDVTTMIPKCSTQLKDVQAYFKVNTDDYVLNSKAFNKPLTITKEVFDLN NVLYGKYKKFQKGYLTATGDNVGYTHAVNVWIKFCMDFLNSYDSTCI YDFSSLKPESYLSLDAFYQDANLLLYKLSFARASVSYINQLVEEGKMYL FQIYNKDFSEYSKGTPNMHTLYWKALFDERNLADVVYKLNGQAEMFY RKKSIENTHPTHPANHPILNKNKDNKKKESLFDYDLIKDRRYTVDKFMF HVPITMNFKSVGSENINQDVKAYLRHADDMHIIGIDRGERHLLYLVVIDL QGNIKEQYSLNEIVNEYNGNTYHTNYHDLLDVREEERLKARQSWQTIEN IKELKEGYLSQVIHKITQLMVRYHAIVVLEDLSKGFMRSRQKVEKQVYQ

	KFEKMLIDKLNYLVDKKTDVSTPGGLLNAYQLTCKSDSSQKLGKQSGF LFYIPAWNTSKIDPVTGFVNLLDTHSLNSKEKIKAFFSKFDAIRYNKDKK WFEFNLDYDKFGKKAEDTRTKWTLCTRGMRIDTFRNKEKNSQWDNQE VDLTTEMKSLLEHYYIDIHGNLKDAISAQTDKAFFTGLLHILKLTLQMRN SITGTETDYLVSPVADENGIFYDSRSCGNQLPENADANGAYNIARKGLM LIEQIKNAEDLNNVKFDISNKAWLNFAQQKPYKNG (SEQ ID NO: 67)
ErCas12a – previously known at Cpf1 *Eubacterium rectale* Ref Seq. WP_119223642.1	MFSAKLISDILPEFVIHNNNYSASEKEEKTQVIKLFSRFATSFKDYFKNRA NCFSANDISSSSCHRIVNDNAEIFFSNALVYRRIVKNLSNDDINKISGDMK DSLKEMSLEEIYSYEKYGEFITQEGISFYNDICGKVNLFMNLYCQKNKEN KNLYKLRKLHKQILCIADTSYEVPYKFESDEEVYQSVNGFLDNISSKHIV ERLRKIGENYNGYNLDKIYIVSKFYESVSQKTYRDWETINTALEIHYNNI LPGNGKSKADKVKKAVKNDLQKSITEINELVSNYKLCPDDNIKAETYIH EISHILNNFEAQELKYNPEIHLVESELKASELKNVLDVIMNAFHWCSVFM TEELVDKDNNFYAELEEIYDEIYPVISLYNLVRNYVTQKPYSTKKIKLNF GIPTLADGWSKSKEYSNNAIILMRDNLYYLGIFNAKNKPDKKIIEGNTSE NKGDYKKMIYNLLPGPNKMIPKVFLSSKTGVETYKPSAYILEGYKQNKH LKSSKDFDITFCHDLIDYFKNCIAIHPEWKNFGFDFSDTSTYEDISGFYRE VELQGYKIDWTYISEKDIDLLQEKGQLYLFQIYNKDFSKKSSGNDNLHT MYLKNLFSEENLKDIVLKLNGEAEIFFRKSSIKNPIIHKKGSILVNRTYEA EEKDQFGNIQIVRKTIPENIYQELYKYFNDKSDKELSDEAAKLKNVVGH HEAATNIVKDYRYTYDKYFLHMPITINFKANKTSFINDRILQYIAKEKDL HVIGIDRGERNLIYVSVIDTCGNIVEQKSFNIVNGYDYQIKLKQQEGARQI ARKEWKEIGKIKEIKEGYLSLVIHEISKMVIKYNAIIAMEDLSYGFKKGRF KVERQVYQKFETMLINKLNYLVFKDISITENGGLLKGYQLTYIPDKLKN VGHQCGCIFYVPAAYTSKIDPTTGFVNIFKFKDLTVDAKREFIKKFDSIRY DSDKNLFCFTFDYNNFITQNTVMSKSSWSVYTYGVRIKRRFVNGRFSNE SDTIDITKDMEKTLEMTDINWRDGHDLRQDIIDYEIVQHIFEIFKLTVQM RNSLSELEDRDYDRLISPVLNENNIFYDSAKAGDALPKDADANGAYCIA LKGLYEIKQITENWKEDGKFSRDKLKISNKDWFDFIQNKRYL (SEQ ID NO: 68)
CsCas12a – previously known at Cpf1 *Clostridium sp. AF34-10BH* Ref Seq. WP_118538418.1	MNYKTGLEDFIGKESLSKTLRNALIPTESTKIHMEEMGVIRDDELRAEKQ QELKEIMDDYYRAFIEEKLGQIQGIQWNSLFQKMEETMEDISVRKDLDKI QNEKRKEICCYFTSDKRFKDLFNAKLITDILPNFIKDNKEYTEEEKAEKE QTRVLFQRFATAFTNYFNQRRNNFSEDNISTAISFRIVNENSEIHLQNMR AFQRIEQQYPEEVCGMEEEYKDMLQEWQMKHIYLVDFYDRVLTQPGIE YYNGICGKINEHMNQFCQKNRINKNDFRMKKLHKQILCKKSSYYEIPFR FESDQEVYDALNEFIKTMKEKEIICRCVHLGQKCDDYDLGKIYISSNKYE QISNALYGSWDTIRKCIKEEYMDALPGKGEKKEEKAEAAAKKEEYRSIA DIDKIISLYGSEMDRTISAKKCITEICDMAGQISTDPLVCNSDIKLLQNKE KTTEIKTILDSFLHVYQWGQTFIVSDIIEKDSYFYSELEDVLEDFEGITTLY NHVRSYVTQKPYSTVKFKLHFGSPTLANGWSQSKEYDNNAILLMRDQK FYLGIFNVRNKPDKQIIKGHEKEEKGDYKKMIYNLLPGPSKMLPKVFITS RSGQETYKPSKHILDGYNEKRHIKSSPKFDLGYCWDLIDYYKECIHKHP DWKNYDFHFSDTKDYEDISGFYREVEMQGYQIKWTYISADEIQKLDEK GQIFLFQIYNKDFSVHSTGKDNLHTMYLKNLFSEENLKDIVLKLNGEAEL FFRKASIKTPVVHKKGSVLVNRSYTQTVGDKEIRVSIPEEYYTEIYNYLN HIGRGKLSTEAQRYLEERKIKSFTATKDIVKNYRYCCDHYFLHLPITINFK AKSDIAVNERTLAYIAKKEDIHIIGIDRGERNLLYISVVDVHGNIREQRSF NIVNGYDYQQKLKDREKSRDAARKNWEEIEKIKELKEGYLSMVIHYIAQ

	LVVKYNAVVAMEDLNYGFKTGRFKVERQVYQKFETMLIEKLHYLVFK DREVCEEGGVLRGYQLTYIPESLKKVGKQCGFIFYVPAGYTSKIDPTTGF VNLFSFKNLTNRESRQDFVGKFDEIRYDRDKKMFEFSFDYNNYIKKGTM LASTKWKVYTNGTRLKRIVVNGKYTSQSMEVELTDAMEKMLQRAGIE YHDGKDLKGQIVEKGIEAEIIDIFRLTVQMRNSRSESEDREYDRLISPVLN DKGEFFDTATADKTLPQDADANGAYCIALKGLYEVKQIKENWKENEQF PRNKLVQDNKTWFDFMQKKRYL (SEQ ID NO: 69)
BhCas12b *Bacillus hisashii* Ref Seq. WP_095142515.1	MATRSFILKIEPNEEVKKGLWKTHEVLNHGIAYYMNILKLIRQEAIYEHH EQDPKNPKKVSKAEIQAELWDFVLKMQKCNSFTHEVDKDEVFNILREL YEELVPSSVEKKGEANQLSNKFLYPLVDPNSQSGKGTASSGRKPRWYNL KIAGDPSWEEEKKKWEEDKKKDPLAKILGKLAEYGLIPLFIPYTDSNEPI VKEIKWMEKSRNQSVRRLDKDMFIQALERFLSWESWNLKVKEEYEKVE KEYKTLEERIKEDIQALKALEQYEKERQEQLLRDTLNTNEYRLSKRGLR GWREIIQKWLKMDENEPSEKYLEVFKDYQRKHPREAGDYSVYEFLSKK ENHFIWRNHPEYPYLYATFCEIDKKKKDAKQQATFTLADPINHPLWVRF EERSGSNLNKYRILTEQLHTEKLKKKLTVQLDRLIYPTESGGWEEKGKV DIVLLPSRQFYNQIFLDIEEKGKHAFTYKDESIKFPLKGTLGGARVQFDR DHLRRYPHKVESGNVGRIYFNMTVNIEPTESPVSKSLKIHRDDFPKVVNF KPKELTEWIKDSKGKKLKSGIESLEIGLRVMSIDLGQRQAAAASIFEVVD QKPDIEGKLFFPIKGTELYAVHRASFNIKLPGETLVKSREVLRKAREDNL KLMNQKLNFLRNVLHFQQFEDITEREKRVTKWISRQENSDVPLVYQDEL IQIRELMYKPYKDWVAFLKQLHKRLEVEIGKEVKHWRKSLSDGRKGLY GISLKNIDEIDRTRKFLLRWSLRPTEPGEVRRLEPGQRFAIDQLNHLNALK EDRLKKMANTIIMHALGYCYDVRKKKWQAKNPACQIILFEDLSNYNPY EERSRFENSKLMKWSRREIPRQVALQGEIYGLQVGEVGAQFSSRFHAKT GSPGIRCSVVTKEKLQDNRFFKNLQREGRLTLDKIAVLKEGDLYPDKGG EKFISLSKDRKCVTTHADINAAQNLQKRFWTRTHGFYKVYCKAYQVDG QTVYIPESKDQKQKIIEEFGEGYFILKDGVYEWVNAGKLKIKKGSSKQSS SELVDSDILKDSFDLASELKGEKLMLYRDPSGNVFPSDKWMAAGVFFG KLERILISKLTNQYSISTIEDDSSKQSM (SEQ ID NO: 70)
ThCas12b *Thermomonas hydrothermalis* Ref Seq. WP_072754838	MSEKTTQRAYTLRLNRASGECAVCQNNSCDCWHDALWATHKAVNRG AKAFGDWLLTLRGGLCHTLVEMEVPAKGNNPPQRPTDQERRDRRVLLA LSWLSVEDEHGAPKEFIVATGRDSADDRAKKVEEKLREILEKRDFQEHEI DAWLQDCGPSLKAHIREDAVWVNRRALFDAAVERIKTLTWEEAWDFL EPFFGTQYFAGIGDGKDKDDAEGPARQGEKAKDLVQKAGQWLSARFGI GTGADFMSMAEAYEKIAKWASQAQNGDNGKATIEKLACALRPSEPPTL DTVLKCISGPGHKSATREYLKTLDKKSTVTQEDLNQLRKLADEDARNC RKKVGKKGKKPWADEVLKDVENSCELTYLQDNSPARHREFSVMLDHA ARRVSMAHSWIKKAEQRRRQFESDAQKLKNLQERAPSAVEWLDRFCES RSMTTGANTGSGYRIRKRAIEGWSYVVQAWAEASCDTEDKRIAAARKV QADPEIEKFGDIQLFEALAADEAICVWRDQEGTQNPSILIDYVTGKTAEH NQKRFKVPAYRHPDELRHPVFCDFGNSRWSIQFAIHKEIRDRDKGAKQD TRQLQNRHGLKMRLWNGRSMTDVNLHWSSKRLTADLALDQNPNPNPT EVTRADRLGRAASSAFDHVKIKNVFNEKEWNGRLQAPRAELDRIAKLE EQGKTEQAEKLRKRLRWYVSFSPCLSPSGPFIVYAGQHNIQPKRSGQYA PHAQANKGRARLAQLILSRLPDLRILSVDLGHRFAAACAVWETLSSDAF RREIQGLNVLAGGSGEGDLFLHVEMTGDDGKRRTVVYRRIGPDQLLDN TPHPAPWARLDRQFLIKLQGEDEGVREASNEELWTVHKLEVEVGRTVP LIDRMVRSGFGKTEKQKERLKKLRELGWISAMPNEPSAETDEKEGEIRSI

	SRSVDELMSSALGTLRLALKRHGNRARIAFAMTADYKPMPGGQKYYFH EAKEASKNDDETKRRDNQIEFLQDALSLWHDLFSSPDWEDNEAKKLWQ NHIATLPNYQTPEEISAELKRVERNKKRKENRDKLRTAAKALAENDQLR QHLHDTWKERWESDDQQWKERLRSLKDWIFPRGKAEDNPSIRHVGGLS ITRINTISGLYQILKAFKMRPEPDDLRKNIPQKGDDELENFNRRLLEARDR LREQRVKQLASRIIEAALGVGRIKIPKNGKLPKRPRTTVDTPCHAVVIESL KTYRPDDLRTRRENRQLMQWSSAKVRKYLKEGCELYGLHFLEVPANYT SRQCSRTGLPGIRCDDVPTGDFLKAPWWRRAINTAREKNGGDAKDRFL VDLYDHLNNLQSKGEALPATVRVPRQGGNLFIAGAQLDDTNKERRAIQ ADLNAAANIGLRALLDPDWRGRWWYVPCKDGTSEPALDRIEGSTAFND VRSLPTGDNSSRRAPREIENLWRDPSGDSLESGTWSPTRAYWDTVQSRV IELLRRHAGLPTS (SEQ ID NO: 71)
LsCas12b *Laceyella sacchari* WP_13222 1894.1	MSIRSFKLKLKTKSGVNAEQLRRGLWRTHQLINDGIAYYMNWLVLLRQ EDLFIRNKETNEIEKRSKEEIQAVLLERVHKQQQRNQWSGEVDEQTLLQ ALRQLYEEIVPSVIGKSGNASLKARFFLGPLVDPNNKTTKDVSKSGPTPK WKKMKDAGDPNWVQEYEKYMAERQTLVRLEEMGLIPLFPMYTDEVG DIHWLPQASGYTRTWDRDMFQQAIERLLSWESWNRRVRERRAQFEKKT HDFASRFSESDVQWMNKLREYEAQQEKSLEENAFAPNEPYALTKKALR GWERVYHSWMRLDSAASEEAYWQEVATCQTAMRGEFGDPAIYQFLAQ KENHDIWRGYPERVIDFAELNHLQRELRRAKEDATFTLPDSVDHPLWVR YEAPGGTNIHGYDLVQDTKRNLTLILDKFILPDENGSWHEVKKVPFSLA KSKQFHRQVWLQEEQKQKKREVVFYDYSTNLPHLGTLAGAKLQWDRN FLNKRTQQQIEETGEIGKVFFNISVDVRPAVEVKNGRLQNGLGKALTVL THPDGTKIVTGWKAEQLEKWVGESGRVSSLGLDSLSEGLRVMSIDLGQ RTSATVSVFEITKEAPDNPYKFFYQLEGTEMFAVHQRSFLLALPGENPPQ KIKQMREIRWKERNRIKQQVDQLSAILRLHKKVNEDERIQAIDKLLQKV ASWQLNEEIATAWNQALSQLYSKAKENDLQWNQAIKNAHHQLEPVVG KQISLWRKDLSTGRQGIAGLSLWSIEELEATKKLLTRWSKRSREPGVVK RIERFETFAKQIQHHINQVKENRLKQLANLIVMTALGYKYDQEQKKWIE VYPACQVVLFENLRSYRFSFERSRRENKKLMEWSHRSIPKLVQMQGELF GLQVADVYAAYSSRYHGRTGAPGIRCHALTEADLRNETNIIHELIEAGFI KEEHRPYLQQGDLVPWSGGELFATLQKPYDNPRILTLHADINAAQNIQK RFWHPSMWFRVNCESVMEGEIVTYVPKNKTVHKKQGKTFRFVKVEGS DVYEWAKWSKNRNKNTFSSITERKPPSSMILFRDPSGTFFKEQEWVEQK TFWGKVQSMIQAYMKKTIVQRMEE (SEQ ID NO: 72)
DtCas12b *Dsulfonatro num thiodismuta ns* *WP_03138 6437*	MVLGRKDDTAELRRALWTTHEHVNLAVAEVERVLLRCRGRSYWTLDR RGDPVHVPESQVAEDALAMAREAQRRNGWPVVGEDEEILLALRYLYEQ IVPSCLLDDLGKPLKGDAQKIGTNYAGPLFDSDTCRRDEGKDVACCGPF HEVAGKYLGALPEWATPISKQEFDGKDASHLRFKATGGDDAFFRVSIEK ANAWYEDPANQDALKNKAYNKDDWKKEKDKGISSWAVKYIQKQLQL GQDPRTEVRRKLWLELGLLPLFIPVFDKTMVGNLWNRLAVRLALAHLL SWESWNHRAVQDQALARAKRDELAALFLGMEDGFAGLREYELRRNESI KQHAFEPVDRPYVVSGRALRSWTRVREEWLRHGDTQESRKNICNRLQD RLRGKFGDPDVFHWLAEDGQEALWKERDCVTSFSLLNDADGLLEKRK GYALMTFADARLHPRWAMYEAPGGSNLRTYQIRKTENGLWADVVLLS PRNESAAVEEKTFNVRLAPSGQLSNVSFDQIQKGSKMVGRCRYQSANQ QFEGLLGGAEILFDRKRIANEQHGATDLASKPGHVWFKLTLDVRPQAPQ GWLDGKGRPALPPEAKHFKTALSNKSKFADQVRPGLRVLSVDLGVRSF AACSVFELVRGGPDQGTYFPAADGRTVDDPEKLWAKHERSFKITLPGEN

	PSRKEEIARRAAMEELRSLNGDIRRLKAILRLSVLQEDDPRTEHLRLFME AIVDDPAKSALNAELFKGFGDDRFRSTPDLWKQHCHFFHDKAEKVVAE RFSRWRTETRPKSSSWQDWRERRGYAGGKSYWAVTYLEAVRGLILRW NMRGRTYGEVNRQDKKQFGTVASALLHHINQLKEDRIKTGADMIIQAA RGFVPRKNGAGWVQVHEPCRLILFEDLARYRFRTDRSRRENSRLMRWS HREIVNEVGMQGELYGLHVDTTEAGFSSRYLASSGAPGVRCRHLVEEDF HDGLPGMHLVGELDWLLPKDKDRTANEARRLLGGMVRPGMLVPWDG GELFATLNAASQLHVIHADINAAQNLQRRFWGRCGEAIRIVCNQLSVDG STRYEMAKAPKARLLGALQQLKNGDAPFHLTSIPNSQKPENSYVMTPTN AGKKYRAGPGEKSSGEEDELALDIVEQAEELAQGRKTFFRDPSGVFFAP DRWLPSEIYWSRIRRRIWQVTLERNSSGRQERAEMDEMPY (SEQ ID NO: 73)

[0340] The prime editors described herein may also comprise Cas12a (Cpf1) (dCpf1) variants that may be used as a guide nucleotide sequence-programmable DNA-binding protein domain. The Cas12a (Cpf1) protein has a RuvC-like endonuclease domain that is similar to the RuvC domain of Cas9 but does not have a HNH endonuclease domain, and the N-terminal of Cas12a (Cpf1) does not have the alfa-helical recognition lobe of Cas9. It was shown in Zetsche *et al.*, *Cell*, 163, 759–771, 2015 (which is incorporated herein by reference) that, the RuvC-like domain of Cas12a (Cpf1) is responsible for cleaving both DNA strands and inactivation of the RuvC-like domain inactivates Cas12a (Cpf1) nuclease activity.

[0341] In some embodiments, the napDNAbp is a single effector of a microbial CRISPR-Cas system. Single effectors of microbial CRISPR-Cas systems include, without limitation, Cas9, Cas12a (Cpf1), Cas12b1 (C2c1), Cas13a (C2c2), and Cas12c (C2c3). Typically, microbial CRISPR-Cas systems are divided into Class 1 and Class 2 systems. Class 1 systems have multisubunit effector complexes, while Class 2 systems have a single protein effector. For example, Cas9 and Cas12a (Cpf1) are Class 2 effectors. In addition to Cas9 and Cas12a (Cpf1), three distinct Class 2 CRISPR-Cas systems (Cas12b1, Cas13a, and Cas12c) have been described by Shmakov et al., "Discovery and Functional Characterization of Diverse Class 2 CRISPR Cas Systems", *Mol. Cell*, 2015 Nov 5; 60(3): 385–397, the entire contents of which are hereby incorporated by reference.

[0342] Effectors of two of the systems, Cas12b1 and Cas12c, contain RuvC-like endonuclease domains related to Cas12a. A third system, Cas13a contains an effector with two predicated HEPN RNase domains. Production of mature CRISPR RNA is tracrRNA-independent, unlike production of CRISPR RNA by Cas12b1. Cas12b1 depends on both CRISPR RNA and tracrRNA for DNA cleavage. Bacterial Cas13a has been shown to possess a unique RNase activity for CRISPR RNA maturation distinct from its RNA-activated single-stranded RNA degradation activity. These RNase functions are different from each other and

from the CRISPR RNA-processing behavior of Cas12a. See, e.g., East-Seletsky, et al., "Two distinct RNase activities of CRISPR-Cas13a enable guide-RNA processing and RNA detection", *Nature,* 2016 Oct 13;538(7624):270-273, the entire contents of which are hereby incorporated by reference. *In vitro* biochemical analysis of Cas13a in *Leptotrichia shahii* has shown that Cas13a is guided by a single CRISPR RNA and can be programed to cleave ssRNA targets carrying complementary protospacers. Catalytic residues in the two conserved HEPN domains mediate cleavage. Mutations in the catalytic residues generate catalytically inactive RNA-binding proteins. See e.g., Abudayyeh et al., "C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector", *Science,* 2016 Aug 5; 353(6299), the entire contents of which are hereby incorporated by reference.

[0343] The crystal structure of *Alicyclobaccillus acidoterrastris* Cas12b1 (AacC2c1) has been reported in complex with a chimeric single-molecule guide RNA (sgRNA). See e.g., Liu et al., "C2c1-sgRNA Complex Structure Reveals RNA-Guided DNA Cleavage Mechanism", *Mol. Cell,* 2017 Jan 19;65(2):310-322, the entire contents of which are hereby incorporated by reference. The crystal structure has also been reported in *Alicyclobacillus acidoterrestris* C2c1 bound to target DNAs as ternary complexes. See e.g., Yang et al., "PAM-dependent Target DNA Recognition and Cleavage by C2C1 CRISPR-Cas endonuclease", *Cell,* 2016 Dec 15;167(7):1814-1828, the entire contents of which are hereby incorporated by reference. Catalytically competent conformations of AacC2c1, both with target and non-target DNA strands, have been captured independently positioned within a single RuvC catalytic pocket, with C2c1-mediated cleavage resulting in a staggered seven-nucleotide break of target DNA. Structural comparisons between C2c1 ternary complexes and previously identified Cas9 and Cpf1 counterparts demonstrate the diversity of mechanisms used by CRISPR-Cas9 systems.

[0344] In some embodiments, the napDNAbp may be a C2c1, a C2c2, or a C2c3 protein. In some embodiments, the napDNAbp is a C2c1 protein. In some embodiments, the napDNAbp is a Cas13a protein. In some embodiments, the napDNAbp is a Cas12c protein. In some embodiments, the napDNAbp comprises an amino acid sequence that is at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring Cas12b1 (C2c1), Cas13a (C2c2), or Cas12c (C2c3) protein. In some embodiments, the napDNAbp is a naturally-occurring Cas12b1 (C2c1), Cas13a (C2c2), or Cas12c (C2c3) protein.

H. Cas9 circular permutants

[0345] In various embodiments, the prime editors disclosed herein may comprise a circular permutant of Cas9.

[0346] The term "circularly permuted Cas9" or "circular permutant" of Cas9 or "CP-Cas9") refers to any Cas9 protein, or variant thereof, that occurs or has been modify to engineered as a circular permutant variant, which means the N-terminus and the C-terminus of a Cas9 protein (e.g., a wild type Cas9 protein) have been topically rearranged. Such circularly permuted Cas9 proteins, or variants thereof, retain the ability to bind DNA when complexed with a guide RNA (gRNA). See, Oakes et al., "Protein Engineering of Cas9 for enhanced function," *Methods Enzymol*, 2014, 546: 491–511 and Oakes et al., "CRISPR-Cas9 Circular Permutants as Programmable Scaffolds for Genome Modification," *Cell*, January 10, 2019, 176: 254-267, each of are incorporated herein by reference. The instant disclosure contemplates any previously known CP-Cas9 or use a new CP-Cas9 so long as the resulting circularly permuted protein retains the ability to bind DNA when complexed with a guide RNA (gRNA).

[0347] Any of the Cas9 proteins described herein, including any variant, ortholog, or naturally occurring Cas9 or equivalent thereof, may be reconfigured as a circular permutant variant.

[0348] In various embodiments, the circular permutants of Cas9 may have the following structure:

N-terminus-[original C-terminus] – [optional linker] – [original N-terminus]-C-terminus.

[0349] As an example, the present disclosure contemplates the following circular permutants of canonical *S. pyogenes* Cas9 (1368 amino acids of UniProtKB - Q99ZW2 (CAS9_STRP1) (numbering is based on the amino acid position in SEQ ID NO: 18)):

N-terminus-[1268-1368]-[optional linker]-[1-1267]-C-terminus;

N-terminus-[1168-1368]-[optional linker]-[1-1167]-C-terminus;

N-terminus-[1068-1368]-[optional linker]-[1-1067]-C-terminus;

N-terminus-[968-1368]-[optional linker]-[1-967]-C-terminus;

N-terminus-[868-1368]-[optional linker]-[1-867]-C-terminus;

N-terminus-[768-1368]-[optional linker]-[1-767]-C-terminus;

N-terminus-[668-1368]-[optional linker]-[1-667]-C-terminus;

N-terminus-[568-1368]-[optional linker]-[1-567]-C-terminus;

N-terminus-[468-1368]-[optional linker]-[1-467]-C-terminus;

N-terminus-[368-1368]-[optional linker]-[1-367]-C-terminus;

N-terminus-[268-1368]-[optional linker]-[1-267]-C-terminus;
N-terminus-[168-1368]-[optional linker]-[1-167]-C-terminus;
N-terminus-[68-1368]-[optional linker]-[1-67]-C-terminus; or
N-terminus-[10-1368]-[optional linker]-[1-9]-C-terminus, or the corresponding circular permutants of other Cas9 proteins (including other Cas9 orthologs, variants, etc).

[0350] In particular embodiments, the circular permuant Cas9 has the following structure (based on *S. pyogenes* Cas9 (1368 amino acids of UniProtKB - Q99ZW2 (CAS9_STRP1) (numbering is based on the amino acid position in SEQ ID NO: 18):
N-terminus-[102-1368]-[optional linker]-[1-101]-C-terminus;
N-terminus-[1028-1368]-[optional linker]-[1-1027]-C-terminus;
N-terminus-[1041-1368]-[optional linker]-[1-1043]-C-terminus;
N-terminus-[1249-1368]-[optional linker]-[1-1248]-C-terminus; or
N-terminus-[1300-1368]-[optional linker]-[1-1299]-C-terminus, or the corresponding circular permutants of other Cas9 proteins (including other Cas9 orthologs, variants, etc).

[0351] In still other embodiments, the circular permuant Cas9 has the following structure (based on *S. pyogenes* Cas9 (1368 amino acids of UniProtKB - Q99ZW2 (CAS9_STRP1) (numbering is based on the amino acid position in SEQ ID NO: 18):
N-terminus-[103-1368]-[optional linker]-[1-102]-C-terminus;
N-terminus-[1029-1368]-[optional linker]-[1-1028]-C-terminus;
N-terminus-[1042-1368]-[optional linker]-[1-1041]-C-terminus;
N-terminus-[1250-1368]-[optional linker]-[1-1249]-C-terminus; or
N-terminus-[1301-1368]-[optional linker]-[1-1300]-C-terminus, or the corresponding circular permutants of other Cas9 proteins (including other Cas9 orthologs, variants, etc).

[0352] In some embodiments, the circular permutant can be formed by linking a C-terminal fragment of a Cas9 to an N-terminal fragment of a Cas9, either directly or by using a linker, such as an amino acid linker. In some embodiments, The C-terminal fragment may correspond to the C-terminal 95% or more of the amino acids of a Cas9 (e.g., amino acids about 1300-1368), or the C-terminal 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% or more of a Cas9 (e.g., any one of SEQ ID NOs: 77-86). The N-terminal portion may correspond to the N-terminal 95% or more of the amino acids of a Cas9 (e.g., amino acids about 1-1300), or the N-terminal 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% or more of a Cas9 (e.g., of SEQ ID NO: 18).

[0353] In some embodiments, the circular permutant can be formed by linking a C-terminal fragment of a Cas9 to an N-terminal fragment of a Cas9, either directly or by using a linker, such as an amino acid linker. In some embodiments, the C-terminal fragment that is rearranged to the N-terminus, includes or corresponds to the C-terminal 30% or less of the amino acids of a Cas9 (*e.g.,* amino acids 1012-1368 of SEQ ID NO: 18). In some embodiments, the C-terminal fragment that is rearranged to the N-terminus, includes or corresponds to the C-terminal 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the amino acids of a Cas9 (*e.g.,* the Cas9 of SEQ ID NO: 18). In some embodiments, the C-terminal fragment that is rearranged to the N-terminus, includes or corresponds to the C-terminal 410 residues or less of a Cas9 (*e.g.,* the Cas9 of SEQ ID NO: 18). In some embodiments, the C-terminal portion that is rearranged to the N-terminus, includes or corresponds to the C-terminal 410, 400, 390, 380, 370, 360, 350, 340, 330, 320, 310, 300, 290, 280, 270, 260, 250, 240, 230, 220, 210, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 residues of a Cas9 (*e.g.,* the Cas9 of SEQ ID NO: 18). In some embodiments, the C-terminal portion that is rearranged to the N-terminus, includes or corresponds to the C-terminal 357, 341, 328, 120, or 69 residues of a Cas9 (e.g., the Cas9 of SEQ ID NO: 18).

[0354] In other embodiments, circular permutant Cas9 variants may be defined as a topological rearrangement of a Cas9 primary structure based on the following method, which is based on *S. pyogenes* Cas9 of SEQ ID NO: 18: (a) selecting a circular permutant (CP) site corresponding to an internal amino acid residue of the Cas9 primary structure, which dissects the original protein into two halves: an N-terminal region and a C-terminal region; (b) modifying the Cas9 protein sequence (e.g., by genetic engineering techniques) by moving the original C-terminal region (comprising the CP site amino acid) to preceed the original N-terminal region, thereby forming a new N-terminus of the Cas9 protein that now begins with the CP site amino acid residue. The CP site can be located in any domain of the Cas9 protein, including, for example, the helical-II domain, the RuvCIII domain, or the CTD domain. For example, the CP site may be located (relative the *S. pyogenes* Cas9 of SEQ ID NO: 18) at original amino acid residue 181, 199, 230, 270, 310, 1010, 1016, 1023, 1029, 1041, 1247, 1249, or 1282. Thus, once relocated to the N-terminus, original amino acid 181, 199, 230, 270, 310, 1010, 1016, 1023, 1029, 1041, 1247, 1249, or 1282 would become the new N-terminal amino acid. Nomenclature of these CP-Cas9 proteins may be referred to as

Cas9-CP^{181}, Cas9-CP^{199}, Cas9-CP^{230}, Cas9-CP^{270}, Cas9-CP^{310}, Cas9-CP^{1010}, Cas9-CP^{1016}, Cas9-CP^{1023}, Cas9-CP^{1029}, Cas9-CP^{1041}, Cas9-CP^{1247}, Cas9-CP^{1249}, and Cas9-CP^{1282}, respectively. This description is not meant to be limited to making CP variants from SEQ ID NO: 18, but may be implemented to make CP variants in any Cas9 sequence, either at CP sites that correspond to these positions, or at other CP sites entireley. This description is not meant to limit the specific CP sites in any way. Virtually any CP site may be used to form a CP-Cas9 variant.

[0355] Exemplary CP-Cas9 amino acid sequences, based on the Cas9 of SEQ ID NO: 18, are provided below in which linker sequences are indicated by underlining and optional methionine (M) residues are indicated in bold. It should be appreciated that the disclosure provides CP-Cas9 sequences that do not include a linker sequence or that include different linker sequences. It should be appreciated that CP-Cas9 sequences may be based on Cas9 sequences other than that of SEQ ID NO: 18 and any examples provided herein are not meant to be limiting. Exempalry CP-Cas9 sequences are as follows:

CP name	Sequence	SEQ ID NO:
CP1012	DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTE ITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW DPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKEL LGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSL FELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKR VILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNL GAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYE TRIDLSQLGGD<u>GGSGGSGGSGGSGGSGGSGG</u>DKKYSIG LAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKN LIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEI FSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVD EVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMI KFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPI NASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFG NLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAP LSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVK LNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYP FLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSE ETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVL PKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQK KAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGV EDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLT LTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGW	SEQ ID NO: 77

CP name	Sequence	SEQ ID NO:
	GRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIH DDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGI LQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYL YYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLL NAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETR QITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVS DFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKY PKLESEFVYG	
CP1028	EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG ETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYS VLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGE LQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQ LFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYN KHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK RYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSGG SGGSGGSGGSGGSGGMDKKYSIGLAIGTNSVGWAVIT DEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAE ATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFF HRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYH LRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLN PDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILS ARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNF KSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEH HQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDG GASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQR TFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKI LTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEV VDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFT VYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTN RKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTY HDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEE RLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIR DKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQ KAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDEL VKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRI EEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTR SDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQR KFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQ ILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQ FYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEF VYGDYKVYDVRKMIAKSEQ	SEQ ID NO: 78

CP name	Sequence	SEQ ID NO:
CP1041	NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGK SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLD EIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDA TLIHQSITGLYETRIDLSQLGGDGGSGGSGGSGGSGGSG GSGGDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVL GNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYT RRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDK KHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKA DLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQL VQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIA QLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQ LSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLS DILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPIL EKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGE LHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLAR GNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIER MTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDY FKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFL DNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDK VMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFL KSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSL HEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPE NIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQIL KEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINR LSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNV PSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERG GLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD ENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYH HAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDV RKMIAKSEQEIGKATAKYFFYS	SEQ ID NO: 79
CP1249	PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL GGDGGSGGSGGSGGSGGSGGSGGMDKKYSIGLAIGTN SVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALL FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEM AKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAY HEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRG HFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASG VDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIAL SLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQI	SEQ ID NO: 80

CP name	Sequence	SEQ ID NO:
	GDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKN GYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNR EDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLK DNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETI TPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPK HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKA IVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLT LFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWG RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGIL QTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLY LYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKD DSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVE TRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSK LVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALI KKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAK YFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVW DKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILP KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAK VEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGY KEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNE LALPSKYVNFLYLASHYEKLKGS	
CP1300	KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVLDATLIHQSITGLYETRIDLSQLGGD<u>GGSGGSGGS</u> <u>GGSGGSGGSGG</u>DKKYSIGLAIGTNSVGWAVITDEYKV PSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEE SFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKK LVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNS DVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLS KSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF DLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAA KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDL TLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQE EFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNG SIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIP YYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGA SAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNEL TKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVS GQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVM	SEQ ID NO: 81

CP name	Sequence	SEQ ID NO:
	GRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIK ELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNR GKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSR MNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGD YKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW DPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKEL LGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSL FELENGRKRMLASAGELQKGNELALPSKYVNFLYLAS HYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKR VILADANLDKVLSAYNKHRD	

[0356] The Cas9 circular permutants that may be useful in the prime editing constructs described herein. Exemplary C-terminal fragments of Cas9, based on the Cas9 of SEQ ID NO: 18, which may be rearranged to an N-terminus of Cas9, are provided below. It should be appreciated that such C-terminal fragments of Cas9 are exemplary and are not meant to be limiting. These exemplary CP-Cas9 fragments have the following sequences:

CP name	Sequence	SEQ ID NO:
CP1012 C-terminal fragment	DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKT EITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD WDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVK ELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKY SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYL ASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFS KRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGL YETRIDLSQLGGD	SEQ ID NO: 82
CP1028 C-terminal fragment	EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG ETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGG FSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYS VLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGE LQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQ LFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYN KHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK RYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 83
CP1041 C-terminal fragment	NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSD KLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGK SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKK DLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS	SEQ ID NO: 84

CP name	Sequence	SEQ ID NO:
	KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLD EIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDA TLIHQSITGLYETRIDLSQLGGD	
CP1249 C-terminal fragment	PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANL DKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFK YFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQL GGD	SEQ ID NO: 85
CP1300 C-terminal fragment	KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTST KEVLDATLIHQSITGLYETRIDLSQLGGD	SEQ ID NO: 86

I. Cas9 variants with modified PAM specificities

[0357] The prime editors of the present disclosure may also comprise Cas9 variants with modified PAM specificities. Some aspects of this disclosure provide Cas9 proteins that exhibit activity on a target sequence that does not comprise the canonical PAM (5′-NGG-3′, where N is A, C, G, or T) at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NGG-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NNG-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NNA-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NNC-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NNT-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NGT-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NGA-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NGC-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NAA-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NAC-3′ PAM sequence at its 3′-end. In some embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NAT-3′ PAM sequence at its 3′-end. In still other embodiments, the Cas9 protein exhibits activity on a target sequence comprising a 5′-NAG-3′ PAM sequence at its 3′-end.

[0358] It should be appreciated that any of the amino acid mutations described herein, (e.g., A262T) from a first amino acid residue (e.g., A) to a second amino acid residue (e.g., T) may also include mutations from the first amino acid residue to an amino acid residue that is similar to (e.g., conserved) the second amino acid residue. For example, mutation of an amino acid with a hydrophobic side chain (e.g., alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan) may be a mutation to a second amino acid with a different hydrophobic side chain (e.g., alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, or tryptophan). For example, a mutation of an alanine to a threonine (e.g., a A262T mutation) may also be a mutation from an alanine to an amino acid that is similar in size and chemical properties to a threonine, for example, serine. As another example, mutation of an amino acid with a positively charged side chain (e.g., arginine, histidine, or lysine) may be a mutation to a second amino acid with a different positively charged side chain (e.g., arginine, histidine, or lysine). As another example, mutation of an amino acid with a polar side chain (e.g., serine, threonine, asparagine, or glutamine) may be a mutation to a second amino acid with a different polar side chain (e.g., serine, threonine, asparagine, or glutamine). Additional similar amino acid pairs include, but are not limited to, the following: phenylalanine and tyrosine; asparagine and glutamine; methionine and cysteine; aspartic acid and glutamic acid; and arginine and lysine. The skilled artisan would recognize that such conservative amino acid substitutions will likely have minor effects on protein structure and are likely to be well tolerated without compromising function. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to a threonine may be an amino acid mutation to a serine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to an arginine may be an amino acid mutation to a lysine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to an isoleucine, may be an amino acid mutation to an alanine, valine, methionine, or leucine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to a lysine may be an amino acid mutation to an arginine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to an aspartic acid may be an amino acid mutation to a glutamic acid or asparagine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to a valine may be an amino acid mutation to an alanine, isoleucine, methionine, or leucine. In some embodiments, any amino of the amino acid mutations provided herein from one amino acid to a glycine may be an amino acid

mutation to an alanine. It should be appreciated, however, that additional conserved amino acid residues would be recognized by the skilled artisan and any of the amino acid mutations to other conserved amino acid residues are also within the scope of this disclosure.

[0359] In some embodiments, the Cas9 protein comprises a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAA-3′ PAM sequence at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 1. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 1. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 1.

[0360] Table 1: NAA PAM Clones

Mutations from wild-type SpCas9 (e.g., SEQ ID NO: 18)
D177N, K218R, D614N, D1135N, P1137S, E1219V, A1320V, A1323D, R1333K
D177N, K218R, D614N, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, G715C, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A367T, K710E, R1114G, D1135N, P1137S, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, D861N, D1135N, K1188R, E1219V, Q1221H, H1264H, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, V743I, R753G, M1021T, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, V743I, R753G, E762G, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, S1274R, A1320V, R1333K
A10T, I322V, S409I, E427G, A589S, R753G, D1135N, E1219V, Q1221H, H1264H, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, E757K, G865G, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, R753G, E757K, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, K599R, M631A, R654L, K673E, V743I, R753G, N758H, E762G, D1135N, D1180G, E1219V, Q1221H, Q1256R, H1264Y, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N869S, N1054D, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, L727I, V743I, R753G, E762G, R859S, N946D, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, N1317T, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, A1323D, R1333K

A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, K1151E, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, N803S, N869S, L921P, Y1016D, G1077D, F1080S, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, E630K, R654L, K673E, V743I, R753G, E762G, Q768H, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, Q768H, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, G1223S, H1264Y, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, N803S, N869S, L921P, Y1016D, G1077D, F1801S, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, V743I, R753G, M1021T, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, M673I, N803S, N869S, G1077D, R1114G, D1135N, V1139A, D1180G, E1219V, Q1221H, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, R1114G, D1135N, E1219V, Q1221H, A1320V, R1333K

[0361] In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 1. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 1.

[0362] In some embodiments, the Cas9 protein exhibits an increased activity on a target sequence that does not comprise the canonical PAM (5′-NGG-3′) at its 3′ end as compared to *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18. In some embodiments, the Cas9 protein exhibits an activity on a target sequence having a 3′ end that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 5-fold increased as compared to the activity of *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18 on the same target sequence. In some embodiments, the Cas9 protein exhibits an activity on a target sequence that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold increased as compared to the activity of

Streptococcus pyogenes as provided by SEQ ID NO: 2 on the same target sequence. In some embodiments, the 3′ end of the target sequence is directly adjacent to an AAA, GAA, CAA, or TAA sequence. In some embodiments, the Cas9 protein comprises a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAC-3′ PAM sequence at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 2. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 2. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 2.

[0363] Table 2: NAC PAM Clones

MUTATIONS FROM WILD-TYPE SPCAS9 (E.G., SEQ ID NO: 18)
T472I, R753G, K890E, D1332N, R1335Q, T1337N
I1057S, D1135N, P1301S, R1335Q, T1337N
T472I, R753G, D1332N, R1335Q, T1337N
D1135N, E1219V, D1332N, R1335Q, T1337N
T472I, R753G, K890E, D1332N, R1335Q, T1337N
I1057S, D1135N, P1301S, R1335Q, T1337N
T472I, R753G, D1332N, R1335Q, T1337N
T472I, R753G, Q771H, D1332N, R1335Q, T1337N
E627K, T638P, K652T, R753G, N803S, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, K652T, R753G, N803S, K959N, R1114G, D1135N, K1156E, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, V647I, R753G, N803S, K959N, G1030R, I1055E, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, E630G, T638P, V647A, G687R, N767D, N803S, K959N, R1114G, D1135N, E1219V, D1332G, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, R1114G, D1135N, E1219V, N1266H, D1332N, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, I1057T, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, M631I, T638P, R753G, N803S, K959N, Y1036H, R1114G, D1135N, E1219V, D1251G, D1332G, R1335Q, T1337N
E627K, T638P, R753G, N803S, V875I, K959N, Y1016C, R1114G, D1135N, E1219V, D1251G, D1332G, R1335Q, T1337N, I1348V
K608R, E627K, T638P, V647I, R654L, R753G, N803S, T804A, K848N, V922A, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, R753G, N803S, V922A, K959N, K1014N, V1015A, R1114G, D1135N, K1156N, E1219V, N1252D, D1332N, R1335Q, T1337N
K608R, E627K, R629G, T638P, V647I, A711T, R753G, K775R, K789E, N803S, K959N, V1015A, Y1036H, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N

K608R, E627K, T638P, V647I, T740A, R753G, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, T740A, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
I670S, K608R, E627K, E630G, T638P, V647I, R653K, R753G, I795L, K797N, N803S, K866R, K890N, K959N, Y1016C, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, T740A, G752R, R753G, K797N, N803S, K948E, K959N, V1015A, Y1016S, R1114G, D1135N, E1219V, N1266H, D1332N, R1335Q, T1337N
I570T, A589V, K608R, E627K, T638P, V647I, R654L, Q716R, R753G, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1207G, E1219V, N1234D, D1332N, R1335Q, T1337N
K608R, E627K, R629G, T638P, V647I, R654L, Q740R, R753G, N803S, K959N, N990S, T995S, V1015A, Y1036D, R1114G, D1135N, E1207G, E1219V, N1234D, N1266H, D1332N, R1335Q, T1337N
I562F, V565D, I570T, K608R, L625S, E627K, T638P, V647I, R654I, G752R, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, N1177S, N1234D, D1332N, R1335Q, T1337N
I562F, I570T, K608R, E627K, T638P, V647I, R753G, E790A, N803S, K959N, V1015A, Y1036H, R1114G, D1135N, D1180E, A1184T, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, E627K, T638P, V647I, R654H, R753G, E790A, N803S, K959N, V1015A, R1114G, D1127A, D1135N, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, L625S, E627K, T638P, V647I, R654I, T703P, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
I570S, K608R, E627K, E630G, T638P, V647I, R653K, R753G, I795L, N803S, K866R, K890N, K959N, Y1016C, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, E627K, T638P, V647I, R654H, R753G, E790A, N803S, K959N, V1016A, R1114G, D1135N, E1219V, K1246E, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, R654L, K673E, R753G, E790A, N803S, K948E, K959N, R1114G, D1127G, D1135N, D1180E, E1219V, N1286H, D1332N, R1335Q, T1337N
K608R, L625S, E627K, T638P, V647I, R654I, I670T, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
E627K, M631V, T638P, V647I, K710E, R753G, N803S, N808D, K948E, M1021L, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N, S1338T, H1349R

[0364] In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 2. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 2.

[0365] In some embodiments, the Cas9 protein exhibits an increased activity on a target sequence that does not comprise the canonical PAM (5′-NGG-3′) at its 3′ end as compared to *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18. In some embodiments, the Cas9 protein exhibits an activity on a target sequence having a 3′ end that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 5-fold increased as compared to the activity of *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18 on

the same target sequence. In some embodiments, the Cas9 protein exhibits an activity on a target sequence that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold increased as compared to the activity of *Streptococcus pyogenes* as provided by SEQ ID NO: 18 on the same target sequence. In some embodiments, the 3′ end of the target sequence is directly adjacent to an AAC, GAC, CAC, or TAC sequence.

[0366] In some embodiments, the Cas9 protein comprises a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAT-3′ PAM sequence at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 3. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 3. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 3.

[0367] Table 3: NAT PAM Clones

MUTATIONS FROM WILD-TYPE SPCAS9 (E.G., SEQ ID NO: 18)
K961E, H985Y, D1135N, K1191N, E1219V, Q1221H, A1320A, P1321S, R1335L
D1135N, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
V743I, R753G, E790A, D1135N, G1218S, E1219V, Q1221H, A1227V, P1249S, N1286K, A1293T, P1321S, D1322G, R1335L, T1339I
F575S, M631L, R654L, V748I, V743I, R753G, D853E, V922A, R1114G D1135N, G1218S, E1219V, Q1221H, A1227V, P1249S, N1286K, A1293T, P1321S, D1322G, R1335L, T1339I
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, N1286K, P1321S, D1322G, R1335L
M631L, R654L, R753G, K797E, D853E, V922A, D1012A, R1114G D1135N, G1218S, E1219V, Q1221H, P1249S, N1317K, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, D596Y, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, Q1256R, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, K710E, V750A, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, K649R, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, K1156E, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, I1057G, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, N1308D, P1321S, D1322G, R1335L

M631L, R654L, R753G, D853E, V922A, R1114G, Y1131C, D1135N, E1150V, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L
M631L, R654L, R664K, R753G, D853E, I1057V, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L
M631L, R654L, R664K, R753G, I1057V, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L

[0368] The above description of various napDNAbps which can be used in connection with the presently disclose prime editors is not meant to be limiting in any way. The prime editors may comprise the canonical SpCas9, or any ortholog Cas9 protein, or any variant Cas9 protein—including any naturally occurring variant, mutant, or otherwise engineered version of Cas9—that is known or which can be made or evolved through a directed evolutionary or otherwise mutagenic process. In various embodiments, the Cas9 or Cas9 varants have a nickase activity, i.e., only cleave of strand of the target DNA sequence. In other embodiments, the Cas9 or Cas9 variants have inactive nucleases, i.e., are "dead" Cas9 proteins. Other variant Cas9 proteins that may be used are those having a smaller molecular weight than the canonical SpCas9 (e.g., for easier delivery) or having modified or rearranged primary amino acid structure (e.g., the circular permutant formats). The prime editors described herein may also comprise Cas9 equivalents, including Cas12a/Cpf1 and Cas12b proteins which are the result of convergent evolution. The napDNAbps used herein (e.g., SpCas9, Cas9 variant, or Cas9 equivalents) may also may also contain various modifications that alter/enhance their PAM specifities. Lastly, the application contemplates any Cas9, Cas9 variant, or Cas9 equivalent which has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.9% sequence identity to a reference Cas9 sequence, such as a references SpCas9 canonical sequences or a reference Cas9 equivalent (e.g., Cas12a/Cpf1).

[0369] In a particular embodiment, the Cas9 variant having expanded PAM capabilities is SpCas9 (H840A) VRQR (SEQ ID NO: 87), which has the following amino acid sequence (with the V, R, Q, R substitutions relative to the SpCas9 (H840A) of SEQ ID NO: 51 being show in bold underline. In addition, the methionine residue in SpCas9 (H840) was removed for SpCas9 (H840A) VRQR):

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIE GDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQ LPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGD

QYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ
QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL
LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH
SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKE
DYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLT
LFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGI
LQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELG
SQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFL
KDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT
KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT
LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGD
YKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGET
GEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD
WDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF
LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNFL
YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSA
YNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**Q**Y**R**STKEVLDATLIHQ
SITGLYETRIDLSQLGGD (SEQ ID NO: 87)

[0370] In another particular embodiment, the Cas9 variant having expanded PAM capabilities is SpCas9 (H840A) VRER, which has the following amino acid sequence (with the V, R, E, R substitutions relative to the SpCas9 (H840A) of SEQ ID NO: 51 being shown in bold underline . In addition, the methionine residue in SpCas9 (H840) was removed for SpCas9 (H840A) VRER):

DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET
AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHE
RHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIE
GDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQ
LPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGD
QYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ
QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDL
LRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLA
RGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH
SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKE
DYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLT
LFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL
DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGI
LQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELG
SQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFL
KDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT
KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT
LKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGD
YKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGET
GEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD
WDPKKYGGF**V**SPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF

LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASA**R**ELQKGNELALPSKYVNFL YLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSA YNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRK**E**Y**R**STKEVLDATLIHQ SITGLYETRIDLSQLGGD (SEQ ID NO: 88)

[0371] In some embodiments, the napDNAbp that functions with a non-canonical PAM sequence is an Argonaute protein. One example of such a nucleic acid programmable DNA binding protein is an Argonaute protein from *Natronobacterium gregoryi* (NgAgo). NgAgo is a ssDNA-guided endonuclease. NgAgo binds 5′ phosphorylated ssDNA of ~24 nucleotides (gDNA) to guide it to its target site and will make DNA double-strand breaks at the gDNA site. In contrast to Cas9, the NgAgo–gDNA system does not require a protospacer-adjacent motif (PAM). Using a nuclease inactive NgAgo (dNgAgo) can greatly expand the bases that may be targeted. The characterization and use of NgAgo have been described in Gao *et al.*, *Nat Biotechnol.*, 2016 Jul;34(7):768-73. PubMed PMID: 27136078; Swarts *et al.*, *Nature*. 507(7491) (2014):258-61; and Swarts *et al.*, *Nucleic Acids Res.* 43(10) (2015):5120-9, each of which is incorporated herein by reference.

[0372] In some embodiments, the napDNAbp is a prokaryotic homolog of an Argonaute protein. Prokaryotic homologs of Argonaute proteins are known and have been described, for example, in Makarova K., et al., "Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements", *Biol Direct.* 2009 Aug 25;4:29. doi: 10.1186/1745-6150-4-29, the entire contents of which is hereby incorporated by reference. In some embodiments, the napDNAbp is a Marinitoga piezophila Argunaute (MpAgo) protein. The CRISPR-associated Marinitoga piezophila Argunaute (MpAgo) protein cleaves single-stranded target sequences using 5′-phosphorylated guides. The 5′ guides are used by all known Argonautes. The crystal structure of an MpAgo-RNA complex shows a guide strand binding site comprising residues that block 5′ phosphate interactions. This data suggests the evolution of an Argonaute subclass with noncanonical specificity for a 5′-hydroxylated guide. See, e.g., Kaya et al., "A bacterial Argonaute with noncanonical guide RNA specificity", *Proc Natl Acad Sci U S A*. 2016 Apr 12;113(15):4057-62, the entire contents of which are hereby incorporated by reference). It should be appreciated that other argonaute proteins may be used, and are within the scope of this disclosure.

[0373] *S*ome aspects of the disclosure provide Cas9 domains that have different PAM specificities. Typically, Cas9 proteins, such as Cas9 from *S. pyogenes* (spCas9), require a canonical NGG PAM sequence to bind a particular nucleic acid region. This may limit the

ability to edit desired bases within a genome. In some embodiments, the base editing fusion proteins provided herein may need to be placed at a precise location, for example where a target base is placed within a 4 base region (*e.g.,* a "editing window"), which is approximately 15 bases upstream of the PAM. See Komor, A.C., *et al.*, "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" *Nature* 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference. Accordingly, in some embodiments, any of the fusion proteins provided herein may contain a Cas9 domain that is capable of binding a nucleotide sequence that does not contain a canonical (*e.g.,* NGG) PAM sequence. Cas9 domains that bind to non-canonical PAM sequences have been described in the art and would be apparent to the skilled artisan. For example, Cas9 domains that bind non-canonical PAM sequences have been described in Kleinstiver, B. P., *et al.*, "Engineered CRISPR-Cas9 nucleases with altered PAM specificities" *Nature* 523, 481-485 (2015); and Kleinstiver, B. P., *et al.*, "Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition" *Nature Biotechnology* 33, 1293-1298 (2015); the entire contents of each are hereby incorporated by reference.

[0374] For example, a napDNAbp domain with altered PAM specificity, such as a domain with at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with wild type *Francisella novicida* Cpf1 (D917, E1006, and D1255) (SEQ ID NO: 74), which has the following amino acid sequence:

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDKY
HQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKD
SEKFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKANSDITDIDEALEIIKSFK
GWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNLPKFLENKAKYESLKDKAPEAIN
YEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEIANFNNYLNQSGITKFNTIIGGK
FVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDD
SDVVTTMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDL
SQQVFDDYSVIGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLA
LEEFNKHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLLQ
ASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFEECYFELA
NIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPDNTAILFIKDDKYYL
GVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNPS
EDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFS
DTQRYNSIDEFYREVENQGYKLTFENISESYIDSVVNQGKLYLFQIYNKDFSAYSKG
RPNLHTLYWKALFDERNLQDVVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNK
DNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDV
HILSIDRGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSAR
KDWKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVEKQV
YQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGKQTGIIYYVPA

GFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDK AAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKELEKLLKDYSIEYGHGECIK AAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQAPKN MPQDADANGAYHIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN (SEQ ID NO: 74)

[0375] An additional napDNAbp domain with altered PAM specificity, such as a domain having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with wild type *Geobacillus thermodenitrificans* Cas9 (SEQ ID NO: 75), which has the following amino acid sequence:

MKYKIGLDIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLARSARR RLRRRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNNDEL ARILLHLAKRRGFRSNRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDPKFS LHKRNKEDNYTNTVARDDLEREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRPFA SKDDIEKKVGFCTFEPKEKRAPKATYTFQSFTVWEHINKLRLVSPGGIRALTDDERR LIYKQAFHKNKITFHDVRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFLELGAYHK IRKAIDSVYGKGAAKSFRPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENL ADKVYDEELIEELLNLSFSKFGHLSLKALRNILPYMEQGEVYSTACERAGYTFTGP KKKQKTVLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARELSQSFD ERRKMQKEQEGNRKKNETAIRQLVEYGLTLNPTGLDIVKFKLWSEQNGKCAYSLQ PIEIERLLEPGYTEVDHVIPYSRSLDDSYTNKVLVLTKENREKGNRTPAEYLGLGSER WQQFETFVLTNKQFSKKKRDRLLRLHYDENEENEFKNRNLNDTRYISRFLANFIRE HLKFADSDDKQKVYTVNGRITAHLRSRWNFNKNREESNLHHAVDAAIVACTTPSDI ARVTAFYQRREQNKELSKKTDPQFPQPWPHFADELQARLSKNPKESIKALNLGNY DNEKLESLQPVFVSRMPKRSITGAAHQETLRRYIGIDERSGKIQTVVKKKLSEIQLD KTGHFPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGELGPIIRTIKII DTTNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPIYTIDMMKGILPNKAIEPN KPYSEWKEMTEDYTFRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYYQTIDSSN GGLSLVSHDNNFSLRSIGSRTLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHSKAG ETIRPL (SEQ ID NO: 75)

[0376] In some embodiments, the nucleic acid programmable DNA binding protein (napDNAbp) is a nucleic acid programmable DNA binding protein that does not require a canonical (NGG) PAM sequence. In some embodiments, the napDNAbp is an argonaute protein. One example of such a nucleic acid programmable DNA binding protein is an Argonaute protein from *Natronobacterium gregoryi* (NgAgo). NgAgo is a ssDNA-guided endonuclease. NgAgo binds 5′ phosphorylated ssDNA of ~24 nucleotides (gDNA) to guide it to its target site and will make DNA double-strand breaks at the gDNA site. In contrast to Cas9, the NgAgo–gDNA system does not require a protospacer-adjacent motif (PAM). Using a nuclease inactive NgAgo (dNgAgo) can greatly expand the bases that may be targeted. The characterization and use of NgAgo have been described in Gao *et al.*, *Nat Biotechnol.*, 34(7): 768-73 (2016), PubMed PMID: 27136078; Swarts *et al.*, *Nature*, 507(7491): 258-61 (2014); and Swarts *et al.*, *Nucleic Acids Res.* 43(10) (2015): 5120-9, each

of which is incorporated herein by reference. The sequence of *Natronobacterium gregoryi* Argonaute is provided in SEQ ID NO: 76.

[0377] The disclosed fusion proteins may comprise a napDNAbp domain having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with wild type *Natronobacterium gregoryi* Argonaute (SEQ ID NO: 76), which has the following amino acid sequence:

MTVIDLDSTTTADELTSGHTYDISVTLTGVYDNTDEQHPRMSLAFEQDNGERRYIT LWKNTTPKDVFTYDYATGSTYIFTNIDYEVKDGYENLTATYQTTVENATAQEVGTT DEDETFAGGEPLDHHLDDALNETPDDAETESDSGHVMTSFASRDQLPEWTLHTYT LTATDGAKTDTEYARRTLAYTVRQELYTDHDAAPVATDGLMLLTPEPLGETPLDLD CGVRVEADETRTLDYTTAKDRLLARELVEEGLKRSLWDDYLVRGIDEVLSKEPVLT CDEFDLHERYDLSVEVGHSGRAYLHINFRHRFVPKLTLADIDDDNIYPGLRVKTTY RPRRGHIVWGLRDECATDSLNTLGNQSVVAYHRNNQTPINTDLLDAIEAADRRVVE TRRQGHGDDAVSFPQELLAVEPNTHQIKQFASDGFHQQARSKTRLSASRCSEKAQA FAERLDPVRLNGSTVEFSSEFFTGNNEQQLRLLYENGESVLTFRDGARGAHPDETFS KGIVNPPESFEVAVVLPEQQADTCKAQWDTMADLLNQAGAPPTRSETVQYDAFSS PESISLNVAGAIDPSEVDAAFVVLPPDQEGFADLASPTETYDELKKALANMGIYSQ MAYFDRFRDAKIFYTRNVALGLLAAAGGVAFTTEHAMPGDADMFIGIDVSRSYPE DGASGQINIAATATAVYKDGTILGHSSTRPQLGEKLQSTDVRDIMKNAILGYQQVT GESPTHIVIHRDGFMNEDLDPATEFLNEQGVEYDIVEIRKQPQTRLLAVSDVQYDTP VKSIAAINQNEPRATVATFGAPEYLATRDGGGLPRPIQIERVAGETDIETLTRQVYLL SQSHIQVHNSTARLPITTAYADQASTHATKGYLVQTGAFESNVGFL (SEQ ID NO: 76)

[0378] In addition, any available methods may be utilized to obtain or construct a variant or mutant Cas9 protein. The term "mutation," as used herein, refers to a substitution of a residue within a sequence, e.g., a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). Mutations can include a variety of categories, such as single base polymorphisms, microduplication regions, indel, and inversions, and is not meant to be limiting in any way. Mutations can include "loss-of-function" mutations which is the normal result of a mutation that reduces or abolishes a protein activity. Most loss-of-function mutations are recessive, because in a heterozygote the second chromosome copy carries an unmutated version of the gene coding for a fully functional protein whose presence compensates for the effect of the

mutation. Mutations also embrace "gain-of-function" mutations, which is one which confers an abnormal activity on a protein or cell that is otherwise not present in a normal condition. Many gain-of-function mutations are in regulatory sequences rather than in coding regions, and can therefore have a number of consequences. For example, a mutation might lead to one or more genes being expressed in the wrong tissues, these tissues gaining functions that they normally lack. Because of their nature, gain-of-function mutations are usually dominant.

[0379] Mutations can be introduced into a reference Cas9 protein using site-directed mutagenesis. Older methods of site-directed mutagenesis known in the art rely on sub-cloning of the sequence to be mutated into a vector, such as an M13 bacteriophage vector, that allows the isolation of single-stranded DNA template. In these methods, one anneals a mutagenic primer (i.e., a primer capable of annealing to the site to be mutated but bearing one or more mismatched nucleotides at the site to be mutated) to the single-stranded template and then polymerizes the complement of the template starting from the 3′ end of the mutagenic primer. The resulting duplexes are then transformed into host bacteria and plaques are screened for the desired mutation. More recently, site-directed mutagenesis has employed PCR methodologies, which have the advantage of not requiring a single-stranded template. In addition, methods have been developed that do not require sub-cloning. Several issues must be considered when PCR-based site-directed mutagenesis is performed. First, in these methods it is desirable to reduce the number of PCR cycles to prevent expansion of undesired mutations introduced by the polymerase. Second, a selection must be employed in order to reduce the number of non-mutated parental molecules persisting in the reaction. Third, an extended-length PCR method is preferred in order to allow the use of a single PCR primer set. And fourth, because of the non-template-dependent terminal extension activity of some thermostable polymerases it is often necessary to incorporate an end-polishing step into the procedure prior to blunt-end ligation of the PCR-generated mutant product.

[0380] Mutations may also be introduced by directed evolution processes, such as phage-assisted continuous evolution (PACE) or phage-assisted noncontinuous evolution (PANCE). The term "phage-assisted continuous evolution (PACE)," as used herein, refers to continuous evolution that employs phage as viral vectors. The general concept of PACE technology has been described, for example, in International PCT Application, PCT/US2009/056194, filed September 8, 2009, published as WO 2010/028347 on March 11, 2010; International PCT Application, PCT/US2011/066747, filed December 22, 2011, published as WO 2012/088381 on June 28, 2012; U.S. Application, U.S. Patent No. 9,023,594, issued May 5, 2015,

International PCT Application, PCT/US2015/012022, filed January 20, 2015, published as WO 2015/134121 on September 11, 2015, and International PCT Application, PCT/US2016/027795, filed April 15, 2016, published as WO 2016/168631 on October 20, 2016, the entire contents of each of which are incorporated herein by reference. Variant Cas9s may also be obtain by phage-assisted non-continuous evolution (PANCE)," which as used herein, refers to non-continuous evolution that employs phage as viral vectors. PANCE is a simplified technique for rapid in vivo directed evolution using serial flask transfers of evolving 'selection phage' (SP), which contain a gene of interest to be evolved, across fresh *E. coli* host cells, thereby allowing genes inside the host *E. coli* to be held constant while genes contained in the SP continuously evolve. Serial flask transfers have long served as a widely-accessible approach for laboratory evolution of microbes, and, more recently, analogous approaches have been developed for bacteriophage evolution. The PANCE system features lower stringency than the PACE system.

[0381] Any of the references noted above which relate to Cas9 or Cas9 equivalents are hereby incorporated by reference in their entireties, if not already stated so.

J. Divided napDNAbp domains for split PE delivery

[0382] In various embodiments, the prime editors described herein may be delivered to cells as two or more fragments which become assembled inside the cell (either by passive assembly, or by active assembly, such as using split intein sequences) into a reconstituted prime editor. In some cases, the self assembly may be passive whereby the two or more prime editor fragments associate inside the cell covalently or non-covalently to reconstitute the prime editor. In other cases, the self-assembly may be catalzyed by dimerization domains installed on each of the fragments. Examples of dimerization domains are described herein. In still other cases, the self-assembly may be catalyzed by split intein sequences installed on each of the prime editor fragments.

[0383] Split PE delivery may be advantageous to address various size constraints of different delivery approaches. For example, delivery approaches may include virus-based delivery methods, messenger RNA-based delivery methods, or RNP-based delivery (ribonucleoprotein-based delivery). And, each of these methods of delivery may be more efficient and/or effective by dividing up the prime editor into smaller pieces. Once inside the cell, the smaller pieces can assemble into a functional prime editor. Depending on the means of splitting, the divided prime editor fragments can be reassembled in a non-covalent manner or a covalent manner to reform the prime editor. In one embodiment, the prime editor can be

split at one or more split sites into two or more fragments. The fragments can be unmodified (other than being split). Once the fragments are delivered to the cell (e.g., by direct delivery of a ribonucleoprotein complex or by nucleic delivery – e.g., mRNA delivery or virus vector based delivery), the fragments can reassociate covalently or non-covalently to reconstitute the prime editor. In another embodiment, the prime editor can be split at one or more split sites into two or more fragments. Each of the fragments can be modified to comprise a dimerization domain, whereby each fragment that is formed is coupled to a dimerization domain. Once delivered or expressed within a cell, the dimerization domains of the different fragments associate and bind to one another, bringing the different prime editor fragments together to reform a functional prime editor. In yet another embodiment, the prime editor fragment may be modified to comprise a split intein. Once delivered or expressed within a cell, the split intein domains of the different fragments associate and bind to one another, and then undergo trans-splicing, which results in the excision of the split-intein domains from each of the fragments, and a concomitant formation of a peptide bond between the fragments, thereby restoring the prime editor.

[0384] In one embodiment, the prime editor can be delivered using a split-intein approach.

[0385] The location of the split site can be positioned between any one or more pair of residues in the prime editor and in any domains therein, including within the napDNAbp domain, the polymerase domain (e.g., RT domain), linker domain that joins the napDNAbp domain and the polymerase domain.

[0386] In one embodiment, depicted in FIG. 66, the prime editor (PE) is divided at a split site within the napDNAbp.

[0387] In certain embodiments, the napDNAbp is a canonical SpCas9 polypeptide of SEQ ID NO: 18, as follows:

SpCas9 *Streptococcus pyogenes* M1 SwissProt Accession No. Q99ZW2 Wild type 1368 AA	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDI LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH AILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN	SEQ ID NO: 18

	FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGM RKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEE NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEV VKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHD AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA KSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPT VAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEK NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPED NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDT TIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	

[0388] In certain embodiments, the SpCas9 is split into two fragments at a split site located between residues 1 and 2, or 2 and 3, or 3 and 4, or 4 and 5, or 5 and 6, or 6 and 7, or 7 and 8, or 8 and 9, or 9 and 10, or between any two pair of residues located anywhere between residues 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of canonical SpCas9 of SEQ ID NO: 18.

[0389] In certain embodiments, a napDNAbp is split into two fragments at a split site that is located at a pair of residue that corresponds to any two pair of residues located anywhere between positions 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of canonical SpCas9 of SEQ ID NO: 18.

[0390] In certain embodiments, the SpCas9 is split into two fragments at a split site located between residues 1 and 2, or 2 and 3, or 3 and 4, or 4 and 5, or 5 and 6, or 6 and 7, or 7 and 8, or 8 and 9, or 9 and 10, or between any two pair of residues located anywhere between residues 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of canonical SpCas9 of SEQ ID NO: 18. In certain embodiments, the

split site is located one or more polypeptide bond sites (i.e., a "split site or split-intein split site"), fused to a split intein, and then delivered to cells as separately-encoded fusion proteins. Once the split-intein fusion proteins (i.e., protein halves) are expressed within a cell, the proteins undergo trans-splicing to form a complete or whole PE with the concomitant removal of the joined split-intein sequences.

[0391] For example, as shown in FIG. 66, the N-terminal extein can be fused to a first split-intein (e.g., N intein) and the C-terminal extein can be fused to a second split-intein (e.g., C intein). The N-terminal extein becomes fused to the C-terminal extein to reform a whole prime editor fusion protein comprising an napDNAbp domain and a polymerase domain (e.g., RT domain) upon the self-association of the N intein and the C intein inside the cell, followed by their self-excision, and the concomitant formation of a peptide bond between the N-terminal extein and C-terminal extein portions of a whole prime editor (PE).

[0392] To take advantage of a split-PE delivery strategy using split-inteins, the prime editor needs to be divided at one or more split sites to create at least two separate halves of a prime editor, each of which may be rejoined inside a cell if each half is fused to a split-intein sequence.

[0393] In certain embodiments, the prime editor is split at a single split site. In certain other embodiments, the prime editor is split at two split sites, or three split sites, or four split sites, or more.

[0394] In a preferred embodiment, the prime editor is split at a single split site to create two separate halves of a prime editor, each of which can be fused to a split intein sequence

[0395] An exemplary split intein is the *Ssp* DnaE intein, which comprises two subunits, namely, DnaE-N and DnaE-C. The two different subunits are encoded by separate genes, namely *dnaE-n* and *dnaE-c*, which encode the DnaE-N and DnaE-C subunits, respectively. DnaE is a naturally occurring split intein in *Synechocytis sp.* PCC6803 and is capable of directing trans-splicing of two separate proteins, each comprising a fusion with either DnaE-N or DnaE-C.

[0396] Additional naturally occurring or engineered split-intein sequences are known in the or can be made from whole-intein sequences described herein or those available in the art. Examples of split-intein sequences can be found in Stevens et al., "A promiscuous split intein with expanded protein engineering applications," PNAS, 2017, Vol.114: 8538-8543; Iwai et al., "Highly efficient protein trans-splicing by a naturally split DnaE intein from Nostc punctiforme, FEBS Lett, 580: 1853-1858, each of which are incorporated herein by reference.

Additional split intein sequences can be found, for example, in WO 2013/045632, WO 2014/055782, WO 2016/069774, and EP2877490, the contents each of which are incorporated herein by reference.

[0397] In addition, protein splicing in trans has been described *in vivo* and *in vitro* (Shingledecker, et al., *Gene* 207:187 (1998), Southworth, et al., *EMBO J.* 17:918 (1998); Mills, et al., *Proc. Natl. Acad. Sci. USA,* 95:3543-3548 (1998); Lew, et al., *J. Biol. Chem.,* 273:15887-15890 (1998); Wu, et al., *Biochim. Biophys. Acta* 35732:1 (1998b), Yamazaki, et al., *J. Am. Chem. Soc.* 120:5591 (1998), Evans, et al., *J. Biol. Chem.* 275:9091 (2000); Otomo, et al., *Biochemistry* 38:16040-16044 (1999); Otomo, et al., *J. Biolmol. NMR* 14:105-114 (1999); Scott, et al., *Proc. Natl. Acad. Sci. USA* 96:13638-13643 (1999)) and provides the opportunity to express a protein as to two inactive fragments that subsequently undergo ligation to form a functional product, e.g., as shown in FIGs. 66 and 67 with regard to the formation of a complete PE fusion protein from two separately-expressed halves.

[0398] In various embodiments described herein, the continuous evolution methods (e.g., PACE) may be used to evolve a first portion of a base editor. A first portion could include a single component or domain, e.g., a Cas9 domain, a deaminase domain, or a UGI domain. The separately evolved component or domain can be then fused to the remaining portions of the base editor within a cell by separately express both the evolved portion and the remaining non-evolved portions with split-intein polypeptide domains. The first portion could more broadly include any first amino acid portion of a base editor that is desired to be evolved using a continuous evolution method described herein. The second portion would in this embodiment refer to the remaining amino acid portion of the base editor that is not evolved using the herein methods. The evolved first portion and the second portion of the base editor could each be expressed with split-intein polypeptide domains in a cell. The natural protein splicing mechanisms of the cell would reassemble the evolved first portion and the non-evolved second portion to form a single fusion protein evolved base editor. The evolved first portion may comprise either the N- or C-terminal part of the single fusion protein. In an analogous manner, use of a second orthogonal trans-splicing intein pair could allow the evolved first portion to comprise an internal part of the single fusion protein.

[0399] Thus, any of the evolved and non-evolved components of the base editors herein described may be expressed with split-intein tags in order to facilitate the formation of a complete base editor comprising the evolved and non-evolved component within a cell.

[0400] The mechanism of the protein splicing process has been studied in great detail (Chong, et al., J. Biol. Chem. 1996, 271, 22159-22168; Xu, M-Q & Perler, F. B. EMBO Journal, 1996, 15, 5146-5153) and conserved amino acids have been found at the intein and extein splicing points (Xu, et al., EMBO Journal, 1994, 13 5517-522). The constructs described herein contain an intein sequence fused to the 5′-terminus of the first gene (e.g., the evolved portion of the base editor). Suitable intein sequences can be selected from any of the proteins known to contain protein splicing elements. A database containing all known inteins can be found on the World Wide Web (Perler, F. B. Nucleic Acids Research, 1999, 27, 346-347). The intein sequence is fused at the 3′ end to the 5′ end of a second gene. For targeting of this gene to a certain organelle, a peptide signal can be fused to the coding sequence of the gene. After the second gene, the intein-gene sequence can be repeated as often as desired for expression of multiple proteins in the same cell. For multi-intein containing constructs, it may be useful to use intein elements from different sources. After the sequence of the last gene to be expressed, a transcription termination sequence must be inserted.In one embodiment, a modified intein splicing unit is designed so that it can both catalyze excision of the exteins from the inteins as well as prevent ligation of the exteins. Mutagenesis of the C-terminal extein junction in the Pyrococcus species GB-D DNA polymerase was found to produce an altered splicing element that induces cleavage of exteins and inteins but prevents subsequent ligation of the exteins (Xu, M-Q & Perler, F. B. EMBO Journal, 1996, 15, 5146-5153). Mutation of serine 538 to either an alanine or glycine induced cleavage but prevented ligation. Mutation of equivalent residues in other intein splicing units should also prevent extein ligation due to the conservation of amino acids at the C-terminal extein junction to the intein. A preferred intein not containing an endonuclease domain is the Mycobacterium xenopi GyrA protein (Telenti, et al. J. Bacteriol. 1997, 179, 6378-6382). Others have been found in nature or have been created artificially by removing the endonuclease domains from endonuclease containing inteins (Chong, et al. J. Biol. Chem. 1997, 272, 15587-15590). In a preferred embodiment, the intein is selected so that it consists of the minimal number of amino acids needed to perform the splicing function, such as the intein from the Mycobacterium xenopi GyrA protein (Telenti, A., et al., J. Bacteriol. 1997, 179, 6378-6382). In an alternative embodiment, an intein without endonuclease activity is selected, such as the intein from the Mycobacterium xenopi GyrA protein or the Saccharaomyces cerevisiae VMA intein that has been modified to remove endonuclease domains (Chong, 1997).Further modification of the intein splicing unit may allow the reaction rate of the cleavage reaction to

be altered allowing protein dosage to be controlled by simply modifying the gene sequence of the splicing unit.

[0401] Inteins can also exist as two fragments encoded by two separately transcribed and translated genes. These so-called split inteins self-associate and catalyze protein- splicing activity in trans. Split inteins have been identified in diverse cyanobacteria and archaea (Caspi et al, Mol Microbiol. 50: 1569-1577 (2003); Choi J. et al, J Mol Biol. 556: 1093-1106 (2006.); Dassa B. et al, Biochemistry. 46:322-330 (2007.); Liu X. and Yang J., J Biol Chem. 275:26315-26318 (2003); Wu H. et al.

[0402] Proc Natl Acad Sci USA. £5:9226-9231 (1998.); and Zettler J. et al, FEBS Letters. 553:909-914 (2009)), but have not been found in eukaryotes thus far. Recently, a bioinformatic analysis of environmental metagenomic data revealed 26 different loci with a novel genomic arrangement. At each locus, a conserved enzyme coding region is interrupted by a split intein, with a freestanding endonuclease gene inserted between the sections coding for intein subdomains. Among them, five loci were completely assembled: DNA helicases (gp41-l, gp41-8); Inosine-5 '-monophosphate dehydrogenase (IMPDH-1); and Ribonucleotide reductase catalytic subunits (NrdA-2 and NrdJ-1). This fractured gene organization appears to be present mainly in phages (Dassa et al, Nucleic Acids Research. 57:2560-2573 (2009)).

[0403] The split intein Npu DnaE was characterized as having the highest rate reported for the protein trans-splicing reaction. In addition, the Npu DnaE protein splicing reaction is considered robust and high-yielding with respect to different extein sequences, temperatures from 6 to 37°C, and the presence of up to 6M Urea (Zettler J. et al, FEBS Letters. 553:909-914 (2009); Iwai I. et al, FEBS Letters 550: 1853-1858 (2006)). As expected, when the Cysl Ala mutation at the N-domain of these inteins was introduced, the initial N to S- acyl shift and therefore protein splicing was blocked. Unfortunately, the C- terminal cleavage reaction was also almost completely inhibited. The dependence of the asparagine cyclization at the C-terminal splice junction on the acyl shift at the N-terminal scissile peptide bond seems to be a unique property common to the naturally split DnaE intein alleles (Zettler J. et al. FEBS Letters. 555:909-914 (2009)).

[0404] The mechanism of protein splicing typically has four steps [29-30]: 1) an N-S or N-O acyl shift at the intein N-terminus, which breaks the upstream peptide bond and forms an ester bond between the N- extein and the side chain of the intein's first amino acid (Cys or Ser); 2) a transesterification relocating the N-extein to the intein C-terminus, forming a new ester bond linking the N-extein to the side chain of the C-extein's first amino acid (Cys, Ser,

or Thr); 3) Asn cyclization breaking the peptide bond between the intein and the C-extein; and 4) a S-N or O-N acyl shift that replaces the ester bond with a peptide bond between the N-extein and C-extein.

[0405] Protein trans-splicing, catalyzed by split inteins, provides an entirely enzymatic method for protein ligation [31]. A split-intein is essentially a contiguous intein (e.g. a mini-intein) split into two pieces named N-intein and C-intein, respectively. The N-intein and C-intein of a split intein can associate non-covalently to form an active intein and catalyze the splicing reaction essentially in same way as a contiguous intein does. Split inteins have been found in nature and also engineered in laboratories [31-35]. As used herein, the term "split intein" refers to any intein in which one or more peptide bond breaks exists between the N-terminal and C-terminal amino acid sequences such that the N-terminal and C-terminal sequences become separate molecules that can non-covalently reassociate, or reconstitute, into an intein that is functional for trans-splicing reactions. Any catalytically active intein, or fragment thereof, may be used to derive a split intein for use in the methods of the invention. For example, in one aspect the split intein may be derived from a eukaryotic intein. In another aspect, the split intein may be derived from a bacterial intein. In another aspect, the split intein may be derived from an archaeal intein. Preferably, the split intein so-derived will possess only the amino acid sequences essential for catalyzing trans-splicing reactions.

[0406] As used herein, the "N-terminal split intein (In)" refers to any intein sequence that comprises an N- terminal amino acid sequence that is functional for trans-splicing reactions. An In thus also comprises a sequence that is spliced out when trans-splicing occurs. An In can comprise a sequence that is a modification of the N-terminal portion of a naturally occurring intein sequence. For example, an In can comprise additional amino acid residues and/or mutated residues so long as the inclusion of such additional and/or mutated residues does not render the In non-functional in trans-splicing. Preferably, the inclusion of the additional and/or mutated residues improves or enhances the trans-splicing activity of the In.

[0407] As used herein, the "C-terminal split intein (Ic)" refers to any intein sequence that comprises a C- terminal amino acid sequence that is functional for trans-splicing reactions. In one aspect, the Ic comprises 4 to 7 contiguous amino acid residues, at least 4 amino acids of which are from the last β-strand of the intein from which it was derived. An Ic thus also comprises a sequence that is spliced out when trans-splicing occurs. An Ic can comprise a sequence that is a modification of the C-terminal portion of a naturally occurring intein sequence. For example, an Ic can comprise additional amino acid residues and/or mutated

residues so long as the inclusion of such additional and/or mutated residues does not render the In non-functional in trans-splicing. Preferably, the inclusion of the additional and/or mutated residues improves or enhances the trans-splicing activity of the Ic.

[0408] In some embodiments of the invention, a peptide linked to an Ic or an In can comprise an additional chemical moiety including, among others, fluorescence groups, biotin, polyethylene glycol (PEG), amino acid analogs, unnatural amino acids, phosphate groups, glycosyl groups, radioisotope labels, and pharmaceutical molecules. In other embodiments, a peptide linked to an Ic can comprise one or more chemically reactive groups including, among others, ketone, aldehyde, Cys residues and Lys residues. The N-intein and C-intein of a split intein can associate non-covalently to form an active intein and catalyze the splicing reaction when an "intein-splicing polypeptide (ISP)" is present. As used herein, "intein-splicing polypeptide (ISP)" refers to the portion of the amino acid sequence of a split intein that remains when the Ic, In, or both, are removed from the split intein. In certain embodiments, the In comprises the ISP. In another embodiment, the Ic comprises the ISP. In yet another embodiment, the ISP is a separate peptide that is not covalently linked to In nor to Ic.

[0409] Split inteins may be created from contiguous inteins by engineering one or more split sites in the unstructured loop or intervening amino acid sequence between the -12 conserved beta-strands found in the structure of mini-inteins [25-28]. Some flexibility in the position of the split site within regions between the beta-strands may exist, provided that creation of the split will not disrupt the structure of the intein, the structured beta-strands in particular, to a sufficient degree that protein splicing activity is lost.

[0410] In protein trans-splicing, one precursor protein consists of an N-extein part followed by the N-intein, another precursor protein consists of the C-intein followed by a C-extein part, and a trans-splicing reaction (catalyzed by the N- and C-inteins together) excises the two intein sequences and links the two extein sequences with a peptide bond. Protein trans-splicing, being an enzymatic reaction, can work with very low (e.g. micromolar) concentrations of proteins and can be carried out under physiological conditions.

[2] Other programmable nucleases

[0411] In various embodiments described herein, the prime editors comprise a napDNAbp, such as a Cas9 protein. These proteins are “programmable” by way of their becoming complexed with a guide RNA (or a PEgRNA, as the case may be), which guides the Cas9 protein to a target site on the DNA which possess a sequence that is complementary to the

spacer portion of the gRNA (or PEgRNA) and also which possesses the required PAM sequence. However, in certain embodiment envisioned here, the napDNAbp may be substituted with a different type of programmable protein, such as a zinc finger nuclease or a transcription activator-like effector nuclease (TALEN).

[0412] FIG. 1J depicts such a variation of prime editing contemplated herein that replaces the napDNAbp (e.g., SpCas9 nickase) with any programmable nuclease domain, such as zinc finger nucleases (ZFN) or transcription activator-like effector nucleases (TALEN). As such, it is contemplated that suitable nucleases do not necessarily need to be "programmed" by a nucleic acid targeting molecule (such as a guide RNA), but rather, may be programmed by defining the specificity of a DNA-binding domain, such as and in particular, a nuclease. Just as in prime editing with napDNAbp moities, it is preferable that such alternative programmable nucleases be modified such that only one strand of a target DNA is cut. In other words, the programmable nucleases should function as nickases, preferably. Once a programmable nuclease is selected (e.g., a ZFN or a TALEN), then additional functionalities may be engineered into the system to allow it to operate in accordance with a prime editing-like mechanism. For example, the programmable nucleases may be modified by coupling (e.g., via a chemical linker) an RNA or DNA extension arm thereto, wherein the extension arm comprises a primer binding site (PBS) and a DNA synthesis template. The programmable nuclease may also be coupled (e.g., via a chemical or amino acid linker) to a polymerase, the nature of which will depend upon whether the extension arm is DNA or RNA. In the case of an RNA extension arm, the polymerase can be an RNA-dependent DNA polymerase (e.g., reverse transcriptase). In the case of a DNA extension arm, the polymerase can be a DNA-dependent DNA polymerase (e.g., a prokaryotic polymerase, including Pol I, Pol II, or Pol III, or a eukaryotic polymerase, including Pol a, Pol b, Pol g, Pol d, Pol e, or Pol z). The system may also include other functionalities added as fusions to the programmable nucleases, or added *in trans* to facilitate the reaction as a whole (e.g., (a) a helicase to unwind the DNA at the cut site to make the cut strand with the 3' end available as a primer, (b) a FEN1 to help remove the endogenous strand on the cut strand to drive the reaction towards replacement of the endogenous strand with the synthesized strand, or (c) a nCas9:gRNA complex to create a second site nick on the opposite strand, which may help drive the integration of the synthesize repair through favored cellular repair of the non-edited strand). In an analogous manner to prime editing with a napDNAbp, such a complex with an otherwise programmable nuclease could be used to synthesize and then install a newly

synthesized replacement strand of DNA carrying an edit of interest permanently into a target site of DNA.

[0413] Suitable alternative programmable nucleases are well known in the art which may be used in place of a napDNAbp:gRNA complex to construct an alternative prime editor system that can be programmed to selectively bind a target site of DNA, and which can be further modified in the manner described above to co-localize a polymerase and an RNA or DNA extension arm comprising a primer binding site and a DNA synthesis template to specific nick site . For example, and as represented in FIG. 1J, Transcription Activator-Like Effector Nucleases (TALENs) may be used as the programmable nuclease in the prime editing methods and compositions of matter described herein. TALENS are artificial restriction enzymes generated by fusing the TAL effector DNA binding domain to a DNA cleavage domain. These reagents enable efficient, programmable, and specific DNA cleavage and represent powerful tools for genome editing *in situ*. Transcription activator-like effectors (TALEs) can be quickly engineered to bind practically any DNA sequence. The term TALEN, as used herein, is broad and includes a monomeric TALEN that can cleave double stranded DNA without assistance from another TALEN. The term TALEN is also used to refer to one or both members of a pair of TALENs that are engineered to work together to cleave DNA at the same site. TALENs that work together may be referred to as a left-TALEN and a right-TALEN, which references the handedness of DNA. See U.S. Ser. No. 12/965,590; U.S. Ser. No. 13/426,991 (U.S. Pat. No. 8,450,471); U.S. Ser. No. 13/427,040 (U.S. Pat. No. 8,440,431); U.S. Ser. No. 13/427,137 (U.S. Pat. No. 8,440,432); and U.S. Ser. No. 13/738,381, all of which are incorporated by reference herein in their entirety. In addition, TALENS are described in WO 2015/027134, US 9,181,535, Boch et al., "Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors", Science, vol. 326, pp. 1509-1512 (2009), Bogdanove et al., TAL Effectors: Customizable Proteins for DNA Targeting, Science, vol. 333, pp. 1843-1846 (2011), Cade et al., "Highly efficient generation of heritable zebrafish gene mutations using homo- and heterodimeric TALENs", Nucleic Acids Research, vol. 40, pp. 8001-8010 (2012), and Cermak et al., "Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting", Nucleic Acids Research, vol. 39, No. 17, e82 (2011), each of which are incorporated herein by reference.

[0414] As represented in FIG. 1J, zinc finger nucleases may also be used as alternative programmable nucleases for use in prime editing in place of napDNAbps, such as Cas9

nickases. Like with TALENS, the ZFN proteins may be modified such that they function as nickases, i.e., engineering the ZFN such that it cleaves only one strand of the target DNA in a manner similar to the napDNAbp used with the prime editors described herein. ZFN proteins have been extensively described in the art, for example, in Carroll et al., "Genome Engineering with Zinc-Finger Nucleases," *Genetics*, Aug 2011, Vol. 188: 773-782; Durai et al., "Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells," *Nucleic Acids Res*, 2005, Vol. 33: 5978-90; and Gaj et al., "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering," *Trends Biotechnol.* 2013, Vol.31: 397-405, each of which are incorporated herein by reference in their entireties.

[3] Polymerases (e.g., reverse transcriptases)

[0415] In various embodiments, the prime editor (PE) system disclosed herein includes a polymerase (e.g., DNA-dependent DNA polymerase or RNA-dependent DNA polymerase, such as, reverse transcriptase), or a variant thereof, which can be provided as a fusion protein with a napDNAbp or other programmable nuclease, or provide *in trans*.

[0416] Any polymerase may be used in the prime editors dislosed herein. The polymerases may be wild type polymerases, functional fragments, mutants, variants, or truncated variants, and the like. The polymerases may include wild type polymerases from eukaryotic, prokaryotic, archael, or viral organisms, and/or the polymerases may be modified by genetic engineering, mutagenesis, directed evolution-based processes. The polymerases may include T7 DNA polymerase, T5 DNA polymerase, T4 DNA polymerase, Klenow fragment DNA polymerase, DNA polymerase III and the like. The polymerases may also be thermostable, and may include Taq, Tne, Tma, Pfu, Tfl, Tth, Stoffel fragment, VENT® and DEEPVENT® DNA polymerases, KOD, Tgo, JDF3, and mutants, variants and derivatives thereof (see U.S. Pat. No. 5,436,149; U.S. Pat. No. 4,889,818; U.S. Pat. No. 4,965,185; U.S. Pat. No. 5,079,352; U.S. Pat. No. 5,614,365; U.S. Pat. No. 5,374,553; U.S. Pat. No. 5,270,179; U.S. Pat. No. 5,047,342; U.S. Pat. No. 5,512,462; WO 92/06188; WO 92/06200; WO 96/10640; Barnes, W. M., Gene 112:29-35 (1992); Lawyer, F. C., et al., PCR Meth. Appl. 2:275-287 (1993); Flaman, J.-M, et al., Nuc. Acids Res. 22(15):3259-3260 (1994), each of which are incorporated by reference). For synthesis of longer nucleic acid molecules (e.g, nucleic acid molecules longer than about 3-5 Kb in length), at least two DNA polymerases can be employed. In certain embodiments, one of the polymerases can be substantially lacking a 3' exonuclease activity and the other may have a 3' exonuclease activity. Such pairings may

include polymerases that are the same or different. Examples of DNA polymerases substantially lacking in 3' exonuclease activity include, but are not limited to, Taq, Tne(exo-), Tma(exo-), Pfu(exo-), Pwo(exo-), exo-KOD and Tth DNA polymerases, and mutants, variants and derivatives thereof.

[0417] Preferably, the polymerase usable in the prime editors disclosed herein are "template-dependent" polymerase (since the polymerases are intended to rely on the DNA synthesis template to specify the sequence of the DNA strand under synthesis during prime editing. As used herein, the term "template DNA molecule" refers to that strand of a nucleic acid from which a complementary nucleic acid strand is synthesized by a DNA polymerase, for example, in a primer extension reaction of the DNA synthesis template of a PEgRNA.

[0418] As used herein, the term "template dependent manner" is intended to refer to a process that involves the template dependent extension of a primer molecule (e.g., DNA synthesis by DNA polymerase). The term "template dependent manner" refers to polynucleotide synthesis of RNA or DNA wherein the sequence of the newly synthesized strand of polynucleotide is dictated by the well-known rules of complementary base pairing (see, for example, Watson, J. D. et al., In: Molecular Biology of the Gene, 4th Ed., W. A. Benjamin, Inc., Menlo Park, Calif. (1987)). The term "complementary" refers to the broad concept of sequence complementarity between regions of two polynucleotide strands or between two nucleotides through base-pairing. It is known that an adenine nucleotide is capable of forming specific hydrogen bonds ("base pairing") with a nucleotide which is thymine or uracil. Similarly, it is known that a cytosine nucleotide is capable of base pairing with a guanine nucleotide. As such, in the case of prime editing, it can be said that the single strand of DNA synthesized by the polymerase of the prime editor against the DNA synthesis template is said to be "complementary" to the sequence of the DNA synthesis template.

A. Exemplary polymerases

[0419] In various embodiments, the prime editors described herein comprise a polymerase. The disclosure contemplates any wild type polymerase obtained from any naturally-occurring organism or virus, or obtained from a commercial or non-commercial source. In addition, the polymerases usable in the prime editors of the disclosure can include any naturally-occuring mutant polymerase, engineered mutant polymerase, or other variant polymerase, including truncated variants that retain function. The polymerases usable herein may also be engineered to contain specific amino acid substitutions, such as those specifically disclosed herein. In certain preferred embodiments, the polymerases usable in the prime editors of the

disclosure are template-based polymerases, i.e., they synthesize nucleotide sequences in a template-dependent manner.

[0420] A polymerase is an enzyme that synthesizes a nucleotide strand and which may be used in connection with the prime editor systems described herein. The polymerases are preferrably "template-dependent" polymerases (i.e., a polymerase which synthesizes a nucleotide strand based on the order of nucleotide bases of a template strand). In certain configurations, the polymerases can also be a "template-independent" (i.e., a polymerase which synthesizes a nucleotide strand without the requirement of a template strand). A polymerase may also be further categorized as a "DNA polymerase" or an "RNA polymerase." In various embodiments, the prime editor system comprises a DNA polymerase. In various embodiments, the DNA polymerase can be a "DNA-dependent DNA polymerase" (i.e., whereby the template molecule is a strand of DNA). In such cases, the DNA template molecule can be a PEgRNA, wherein the extension arm comprises a strand of DNA. In such cases, the PEgRNA may be referred to as a chimeric or hybrid PEgRNA which comprises an RNA portion (i.e., the guide RNA components, including the spacer and the gRNA core) and a DNA portion (i.e., the extension arm). In various other embodiments, the DNA polymerase can be an "RNA-dependent DNA polymerase" (i.e., whereby the template molecule is a strand of RNA). In such cases, the PEgRNA is RNA, i.e., including an RNA extension. The term "polymerase" may also refer to an enzyme that catalyzes the polymerization of nucleotide (i.e., the polymerase activity). Generally, the enzyme will initiate synthesis at the 3'-end of a primer annealed to a polynucleotide template sequence (e.g., such as a primer sequence annealed to the primer binding site of a PEgRNA), and will proceed toward the 5' end of the template strand. A "DNA polymerase" catalyzes the polymerization of deoxynucleotides. As used herein in reference to a DNA polymerase, the term DNA polymerase includes a "functional fragment thereof". A "functional fragment thereof" refers to any portion of a wild-type or mutant DNA polymerase that encompasses less than the entire amino acid sequence of the polymerase and which retains the ability, under at least one set of conditions, to catalyze the polymerization of a polynucleotide. Such a functional fragment may exist as a separate entity, or it may be a constituent of a larger polypeptide, such as a fusion protein.

[0421] In some embodiments, the polymerases can be from bacteriophage. Bacteriophage DNA polymerases are generally devoid of 5' to 3' exonuclease activity, as this activity is encoded by a separate polypeptide. Examples of suitable DNA polymerases are T4, T7, and

phi29 DNA polymerase. The enzymes available commercially are: T4 (available from many sources e.g., Epicentre) and T7 (available from many sources, e.g. Epicentre for unmodified and USB for 3' to 5' exo T7 "Sequenase" DNA polymerase).

[0422] The other embodiments, the polymerases are archaeal polymerases. There are 2 different classes of DNA polymerases which have been identified in archaea: 1. Family B/pol I type (homologs of Pfu from *Pyrococcus furiosus*) and 2. pol II type (homologs of *P. furiosus* DP1/DP2 2-subunit polymerase). DNA polymerases from both classes have been shown to naturally lack an associated 5' to 3' exonuclease activity and to possess 3' to 5' exonuclease (proofreading) activity. Suitable DNA polymerases (pol I or pol II) can be derived from archaea with optimal growth temperatures that are similar to the desired assay temperatures.

[0423] Thermostable archaeal DNA polymerases are isolated from *Pyrococcus* species (furiosus, species GB-D, w*oesii, abysii, horikoshii*), *Thermococcus* species (kodakaraensis KOD1, litoralis, species 9 degrees North-7, species JDF-3, gorgonarius), *Pyrodictium occultum*, and *Archaeoglobus fulgidus*.

[0424] Polymerases may also be from eubacterial species. There are 3 classes of eubacterial DNA polymerases, pol I, II, and III. Enzymes in the Pol I DNA polymerase family possess 5' to 3' exonuclease activity, and certain members also exhibit 3' to 5' exonuclease activity. Pol II DNA polymerases naturally lack 5' to 3' exonuclease activity, but do exhibit 3' to 5' exonuclease activity. Pol III DNA polymerases represent the major replicative DNA polymerase of the cell and are composed of multiple subunits. The pol III catalytic subunit lacks 5' to 3' exonuclease activity, but in some cases 3' to 5' exonuclease activity is located in the same polypeptide.

[0425] There are a variety of commercially available Pol I DNA polymerases, some of which have been modified to reduce or abolish 5' to 3' exonuclease activity.

[0426] Suitable thermostable pol I DNA polymerases can be isolated from a variety of thermophilic eubacteria, including *Thermus* species and *Thermotoga maritima* such as *Thermus aquaticus* (Taq), *Thermus thermophilus* (Tth) and *Thermotoga maritima* (Tma UlTma).

[0427] Additional eubacteria related to those listed above are described in Thermophilic Bacteria (Kristjansson, J. K., ed.) CRC Press, Inc., Boca Raton, Fla., 1992.

[0428] The invention further provides for chimeric or non-chimeric DNA polymerases that are chemically modified according to methods disclosed in U.S. Pat. Nos. 5,677,152,

6,479,264 and 6,183,998, the contents of which are hereby incorporated by reference in their entirety.

[0429] Additional archaea DNA polymerases related to those listed above are described in the following references: Archaea: A Laboratory Manual (Robb, F. T. and Place, A. R., eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1995 and Thermophilic Bacteria (Kristjansson, J. K., ed.) CRC Press, Inc., Boca Raton, Fla., 1992.

B. Exemplarly reverse transcriptases

[0430] In various embodiments, the prime editors described herein comprise a reverse transcriptase as the polymerase. The disclosure contemplates any wild type reverse transcriptase obtained from any naturally-occurring organim or virus, or obtained from a commercial or non-commercial source. In addition, the reverse transcriptases usable in the prime editors of the disclosure can include any naturally-occuring mutant RT, engineered mutant RT, or other variant RT, including truncated variants that retain function. The RTs may also be engineered to contain specific amino acid substitutions, such as those specifically disclosed herein.

[0431] Reverse transcriptases are multi-functional enzymes typically with three enzymatic activities including RNA- and DNA-dependent DNA polymerization activity, and an RNaseH activity that catalyzes the cleavage of RNA in RNA-DNA hybrids. Some mutants of reverse transcriptases have disabled the RNaseH moiety to prevent unintended damage to the mRNA. These enzymes that synthesize complementary DNA (cDNA) using mRNA as a template were first identified in RNA viruses. Subsequently, reverse transcriptases were isolated and purified directly from virus particles, cells or tissues. (e.g., see Kacian et al., 1971, Biochim. Biophys. Acta 46: 365-83; Yang et al., 1972, Biochem. Biophys. Res. Comm. 47: 505-11; Gerard et al., 1975, J. Virol. 15: 785-97; Liu et al., 1977, Arch. Virol. 55 187-200; Kato et al., 1984, J. Virol. Methods 9: 325-39; Luke et al., 1990, Biochem. 29: 1764-69 and Le Grice et al., 1991, J. Virol. 65: 7004-07, each of which are incorporated by reference). More recently, mutants and fusion proteins have been created in the quest for improved properties such as thermostability, fidelity and activity. Any of the wild type, variant, and/or mutant forms of reverse transcriptase which are known in the art or which can be made using methods known in the art are contemplated herein.

[0432] The reverse transcriptase (RT) gene (or the genetic information contained therein) can be obtained from a number of different sources. For instance, the gene may be obtained from eukaryotic cells which are infected with retrovirus, or from a number of plasmids which

contain either a portion of or the entire retrovirus genome. In addition, messenger RNA-like RNA which contains the RT gene can be obtained from retroviruses. Examples of sources for RT include, but are not limited to, Moloney murine leukemia virus (M-MLV or MLVRT); human T-cell leukemia virus type 1 (HTLV-1); bovine leukemia virus (BLV); Rous Sarcoma Virus (RSV); human immunodeficiency virus (HIV); yeast, including Saccharomyces, Neurospora, Drosophila; primates; and rodents. See, for example, Weiss, et al., U.S. Pat. No. 4,663,290 (1987); Gerard, G. R., DNA:271-79 (1986); Kotewicz, M. L., et al., Gene 35:249-58 (1985); Tanese, N., et al., Proc. Natl. Acad. Sci. (USA):4944-48 (1985); Roth, M. J., at al., J. Biol. Chem. 260:9326-35 (1985); Michel, F., et al., Nature 316:641-43 (1985); Akins, R. A., et al., Cell 47:505-16 (1986), EMBO J. 4:1267-75 (1985); and Fawcett, D. F., Cell 47:1007-15 (1986) (each of which are incorporated herein by reference in their entireties).

Wild type RTs

[0433] Exemplary enzymes for use with the herein disclosed prime editors can include, but are not limited to, M-MLV reverse transcriptase and RSV reverse transcriptase. Enzymes having reverse transcriptase activity are commercially available. In certain embodiments, the reverse transcriptase provided *in trans* to the other components of the prime editor (PE) system. That is, the reverse transcriptase is expressed or otherwise provided as an individual component, i.e., not as a fusion protein with a napDNAbp.

[0434] A person of ordinary skill in the art will recognize that wild type reverse transcriptases, including but not limited to, Moloney Murine Leukemia Virus (M-MLV); Human Immunodeficiency Virus (HIV) reverse transcriptase and avian Sarcoma-Leukosis Virus (ASLV) reverse transcriptase, which includes but is not limited to Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Avian Erythroblastosis Virus (AEV) Helper Virus MCAV reverse transcriptase, Avian Myelocytomatosis Virus MC29 Helper Virus MCAV reverse transcriptase, Avian Reticuloendotheliosis Virus (REV-T) Helper Virus REV-A reverse transcriptase, Avian Sarcoma Virus UR2 Helper Virus UR2AV reverse transcriptase, Avian Sarcoma Virus Y73 Helper Virus YAV reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, and Myeloblastosis Associated Virus (MAV) reverse transcriptase may be suitably used in the subject methods and composition described herein.

[0435] Exemplary wild type RT enzymes are as follows:

DESCRIPTION	SEQUENCE
REVERSE TRANSCRIPTASE (M-MLV RT) WILD TYPE MOLONEY MURINE LEUKEMIA VIRUS USED IN PE1 (PRIME EDITOR 1 FUSION PROTEIN DISCLOSED HEREIN)	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLA VRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGIL VPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTV PNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEW RDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHP DLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAK KAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPR QLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQ KLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGK LTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLT DQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKA LPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIH GEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQ KGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP(SEQ ID NO: 89)
REVERSE TRANSCRIPTASE MOLONEY MURINE LEUKEMIA VIRUS REF SEQ. AAA66622.1	AFPLERPDWDYTTQAGRNHLVHYRQLLLAGLQNAGRSPTNL AKVKGITQGPNESPSAFLERLKEAYRRYTPYDPEDPGQETNVS MSFIWQSAPDIGRKLGRLEDLKSKTLGDLVREAEKIFNKRETP EEREERIRRETEEKEERRRTVDEQKEKERDRRRHREMSKLLAT VVIGQEQDRQEGERKRPQLDKDQCAYCKEKGHWAKDCPKKP RGPRGPRPQTSLLTLGDXGGQGQDPPPEPRITLKVGGQPVTFL VDTGAQHSVLTQNPGPLSDKSAWVQGATGGKRYRWTTDRKV HLATGKVTHSFLHVPDCPYPLLGRDLLTKLKAQIHFEGSGAQV VGPMGQPLQVLTLNIEDEYRLHETSKEPDVSLGFTWLSDFPQA WAESGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREV NKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRL HPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEAL HRDLADFR(SEQ ID NO: 90)
REVERSE TRANSCRIPTASE FELINE LEUKEMIA VIRUS REF SEQ. NP955579.1	TLQLEEEYRLFEPESTQKQEMDIWLKNFPQAWAETGGMGTAH CQAPVLIQLKATATPISIRQYPMPHEAYQGIKPHIRRMLDQGIL KPCQSPWNTPLLPVKKPGTEDYRPVQDLREVNKRVEDIHPTV PNPYNLLSTLPPSHPWYTVLDLKDAFFCLRLHSESQLLFAFEW RDPEIGLSGQLTWTRLPQGFKNSPTLFDEALHSDLADFRVRYP ALVLLQYVDDLLLAAATRTECLEGTKALLETLGNKGYRASAK KAQICLQEVTYLGYSLKDGQRWLTKARKEAILSIPVPKNSRQV REFLGTAGYCRLWIPGFAELAAPLYPLTRPGTLFQWGTEQQLA FEDIKKALLSSPALGLPDITKPFELFIDENSGFAKGVLVQKLGP WKRPVAYLSKKLDTVASGWPPCLRMVAAIAILVKDAGKLTLG QPLTILTSHPVEALVRQPPNKWLSNARMTHYQAMLLDAERVH FGPTVSLNPATLLPLPSGGNHHDCLQILAETHGTRPDLTDQPLP DADLTWYTDGSSFIRNGEREAGAAVTTESEVIWAAPLPPGTSA QRAELIALTQALKMAEGKKLTVYTDSRYAFATTHVHGEIYRRR GLLTSEGKEIKNKNEILALLEALFLPKRLSIIHCPGHQKGDSPQ AKGNRLADDTAKKAATETHSSLTVL SEQ ID NO: 91)

DESCRIPTION	SEQUENCE
REVERSE TRANSCRIPTASE HIV-1 RT, CHAIN A REF SEQ. ITL3-A	PISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKE GKISKIGPENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDF WEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPLDEDFRKYTA FTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQ NPDIVIYQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTP DKKHQKEPPFLWMGYELHPDKWTVQPIVLPEKDSWTVNDIQ KLVGKLNWASQIYPGIKVRQLXKLLRGTKALTEVIPLTEEAEL ELAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQ EPFKNLKTGKYARMRGAHTNDVKQLTEAVQKITTESIVIWGK TPKFKLPIQKETWETWWTEYWQATWIPEWEFVNTPPLVKLW YQLEKEPIVGAETFYVDGAANRETKLGKAGYVTNRGRQKVV TLTDTTNQKTELQAIYLALQDSGLEVNIVTDSQYALGIIQAQPD QSESELVNQIIEQLIKKEKVYLAWVPAHKGIGGNEQVDKLVSA GIRKV(SEQ ID NO: 92) SEE MARTINELLI ET AL., VIROLOGY, 1990, 174(1): 135-144, WHICH IS INCORPORATED BY REFERENCE
REVERSE TRANSCRIPTASE HIV-1 RT, CHAIN B REF SEQ. ITL3-B	PISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKE GKISKIGPENPYNTPVFAIKKKDSTKWRKLVDFRELNKRTQDF WEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPLDEDFRKYTA FTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFRKQ NPDIVIYQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGLTTP DKKHQKEPPFLWMGYELHPDKWTVQPIVLPEKDSWTVNDIQ KLVGKLNWASQIYPGIKVRQLCKLLRGTKALTEVIPLTEEAELE LAENREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQIYQEP FKNLKTGKYARMRGAHTNDVKQLTEAVQKITTESIVIWGKTP KFKLPIQKETWETWWTEYWQATWIPEWEFVNTPPLVKLWYQ LEKEPIVGAETF(SEQ ID NO: 93) SEE STAMMERS ET AL., J. MOL. BIOL., 1994, 242(4): 586-588, WHICH IS INCORPORATED BY REFERENCE
REVERSE TRANSCRIPTASE ROUS SARCOMA VIRUS RT REF SEQ. ACL14945	TVALHLAIPLKWKPNHTPVWIDQWPLPEGKLVALTQLVEKEL QLGHIEPSLSCWNTPVFVIRKASGSYRLLHDLRAVNAKLVPFG AVQQGAPVLSALPRGWPLMVLDLKDCFFSIPLAEQDREAFAF TLPSVNNQAPARRFQWKVLPQGMTCSPTICQLIVGQILEPLRL KHPSLRMLHYMDDLLLAASSHDGLEAAGEEVISTLERAGFTIS PDKVQKEPGVQYLGYKLGSTYAAPVGLVAEPRIATLWDVQKL VGSLQWLRPALGIPPRLRGPFYEQLRGSDPNEAREWNLDMK MAWREIVQLSTTAALERWDPALPLEGAVARCEQGAIGVLGQG LSTHPRPCLWLFSTQPTKAFTAWLEVLTLLITKLRASAVRTFGK EVDILLLPACFRDELPLPEGILLALRGFAGKIRSSDTPSIFDIARP LHVSLKVRVTDHPVPGPTVFTDASSSTHKGVVVWREGPRWEI KEIADLGASVQQLEARAVAMALLLWPTTPTNVVTDSAFVAKM LLKMGQEGVPSTAAAFILEDALSQRSAMAAVLHVRSHSEVPG FFTEGNDVADSQATFQAYPLREAKDLHTALHIGPRALSKACNI SMQQAREVVQTCPHCNSAPALEAGVNPRGLGPLQIWQTDFTL EPRMAPRSWLAVTVDTASSAIVVTQHGRVTSVAAQHHWATVI AVLGRPKAIKTDNGSCFTSKSTREWLARWGIAHTTGIPGNSQG QAMVERANRLLKDKIRVLAEGDGFMKRIPTSKQGELLAKAM

DESCRIPTION	SEQUENCE
	YALNHFERGENTKTPIQKHWRPTVLTEGPPVKIRIETGEWEKG WNVLVWGRGYAAVKNRDTDKVIWVPSRKVKPDIAQKDEVT KKDEASPLFA(SEQ ID NO: 94) SEE YASUKAWA ET AL., J. BIOCHEM. 2009, 145(3): 315-324, WHICH IS INCORPORATED BY REFERENCE
REVERSE TRANSCRIPTASE CAULIFLOWER MOSAIC VIRUS RT REF SEQ. AGT42196	MMDHLLQKTQIQNQTEQVMNITNPNSIYIKGRLYFKGYKKIEL HCFVDTGASLCIASKFVIPEEHWINAERPIMVKIADGSSITINKV CRDIDLIIAGEIFHIPTVYQQESGIDFIIGNNFCQLYEPFIQFTDRV IFTKDRTYPVHIAKLTRAVRVGTEGFLESMKKRSKTQQPEPVNI STNKIAILSEGRRLSEEKLFITQQRMQKIEELLEKVCSENPLDP NKTKQWMKASIKLSDPSKAIKVKPMKYSPMDREEFDKQIKEL LDLKVIKPSKSPHMAPAFLVNNEAEKRRGKKRMVVNYKAMN KATVGDAYNLPNKDELLTLIRGKKIFSSFDCKSGFWQVLLDQD SRPLTAFTCPQGHYEWNVVPFGLKQAPSIFQRHMDEAFRVFR KFCCVYVDDILVFSNNEEDHLLHVAMILQKCNQHGIILSKKKA QLFKKKINFLGLEIDEGTHKPQGHILEHINKFPDTLEDKKQLQR FLGILTYASDYIPKLAQIRKPLQAKLKENVPWKWTKEDTLYM QKVKKNLQGFPPLHHPLPEEKLIIETDASDDYWGGMLKAIKIN EGTNTELICRYASGSFKAAEKNYHSNDKETLAVINTIKKFSIYL TPVHFLIRTDNTHFKSFVNLNYKGDSKLGRNIRWQAWLSHYS FDVEHIKGTDNHFADFLSREFNRVNS(SEQ ID NO: 95) SEE FARZADFAR ET AL., VIRUS GENES, 2013, 47(2): 347-356, WHICH IS INCORPORATED BY REFERENCE
REVERSE TRANSCRIPTASE KLEBSIELLA PNEUMONIA REF SEQ. RFF81513.1	MKEKISKIDKNFYTDIFIKTSFQNEFEAGGVIPPIAKNQVSTISN KNKTFYSLAHSSPHYSIQTRIEKFLLKNIPLSASSFAFRKERSYL HYLEPHTQNVKYCHLDIVSFFHSIDVNIVRDTFSVYFSDEFLVK EKQSLLDAFMASVTLTAELDGVEKTFIPMGFKSSPSISNIIFRKI DILIQKFCDKNKITYTRYADDLLFSTKKENNILSSTFFINEISSIL SINKFKLNKSKYLYKEGTISLGGYVIENILKDNSSGNIRLSSSKL NPLYKALYEIKKGSSSKHICIKVFNLKLKRFIYKKNKEKFEAKF YSSQLKNKLLGYRSYLLSFVIFHKKYKCINPIFLEKCVFLISEIE SIMNRKF(SEQ ID NO: 96)
REVERSE TRANSCRIPTASE ESCERICHIA COLI RT REF SEQ. TGH57013	MKITSNNVTAVINGKGWHSINWKKCHQHVKTIQTRIAKAACN QQWRTVGRLQRLLVRSFSARALAVKRVTENSGRKTPGVDGQI WSTPESKWEAIFKLRRKGYKPLPLKRVFIPKSNGKKRPLGIPV MLDRAMQALHLLGLEPVSETNADHNSYGFRPARCTADAIQQ VCNMYSSRNASKWVLEGDIKGCFEHISHEWLLENIPMDKQIL RNWLKAGIIEKSIFSKTLSGTPQGGIISPVLANMALDGLERLLQ NRFGRNRLI(SEQ ID NO: 97)
REVERSE TRANSCRIPTASE BACILLUS SUBTILIS RT REF SEQ. QBJ66766	MSKIKINYEKYHIKPFPHFDQRIKVNKKVKENLQNPFYIAAHS FYPFIHYKKISYKFKNGTLSSPKERDIFYSGHMDGYIYKHYGEI LNHKYNNTCIGKGIDHVSLAYRNNKMGKSNIHFAAEVINFISE QQQAFIFVSDFSSYFDSLDHAILKEKLIEVLEEQDKLSKDWWN VFKHITRYNWVEKEEVISDLECTKEKIARDKKSRERYYTPAEF REFRKRVNIKSNDTGVGIPQGTAISAVLANVYAIDLDQKLNQY ALKYGGIYRRYSDDIIMVLPMTSDGQDPSNDHVSFIKSVVKRN

DESCRIPTION	SEQUENCE
	KVTMGDSKTSVLYYANNNIYEDYQRKRESKMDYLGFSFDGM TVKIREKSLFKYYHRTYKKINSINWASVKKEKKVGRKKLYLL YSHLGRNYKGHGNFISYCKKAHAVFEGNKKIESLINQQIKRH WKKIQKRLVDV(SEQ ID NO: 98)
EUBACTERIUM RECTALE GROUP II INTRON RT	DTSNLMEQILSSDNLNRAYLQVVRNKGAEGVDGMKYTELKE HLAKNGETIKGQLRTRKYKPQPARRVEIPKPDGGVRNLGVPT VTDRFIQQAIAQVLTPIYEEQFHDHSYGFRPNRCAQQAILTALN IMNDGNDWIVDIDLEKFFDTVNHDKLMTLIGRTIKDGDVISIV RKYLVSGIMIDDEYEDSIVGTPQGGNLSPLLANIMLNELDKEM EKRGLNFVRYADDCIIMVGSEMSANRVMRNISRFIEEKLGLKV NMTKSKVDRPSGLKYLGFGFYFDPRAHQFKAKPHAKSVAKF KKRMKELTCRSWGVSNSYKVEKLNQLIRGWINYFKIGSMKTL CKELDSRIRYRLRMCIWKQWKTPQNQEKNLVKLGIDRNTARR VAYTGKRIAYVCNKGAVNVAISNKRLASFGLISMLDYYIEKCV TC(SEQ ID NO: 99)
GEOBACILLUS STEAROTHERMOPH ILUS GROUP II INTRON RT	ALLERILARDNLITALKRVEANQGAPGIDGVSTDQLRDYIRAH WSTIHAQLLAGTYRPAPVRRVEIPKPGGGTRQLGIPTVVDRLIQ QAILQELTPIFDPDFSSSSFGFRPGRNAHDAVRQAQGYIQEGYR YVVDMDLEKFFDRVNHDILMSRVARKVKDKRVLKLIRAYLQA GVMIEGVKVQTEEGTPQGGPLSPLLANILLDDLDKELEKRGL KFCRYADDCNIYVKSLRAGQRVKQSIQRFLEKTLKLKVNEEKS AVDRPWKRAFLGFSFTPERKARIRLAPRSIQRLKQRIRQLTNPN WSISMPERIHRVNQYVMGWIGYFRLVETPSVLQTIEGWIRRRL RLCQWLQWKRVRTRIRELRALGLKETAVMEIANTRKGAWRTT KTPQLHQALGKTYWTAQGLKSLTQR(SEQ ID NO: 100)

Variant and error-prone RTs

[0436] Reverse transcriptases are essential for synthesizing complementary DNA (cDNA) strands from RNA templates. Reverse transcriptases are enzymes composed of distinct domains that exhibit different biochemical activities. The enzymes catalyze the synthesis of DNA from an RNA template, as follows: In the presence of an annealed primer, reverse transcriptase binds to an RNA template and initiates the polymerization reaction. RNA-dependent DNA polymerase activity synthesizes the complementary DNA (cDNA) strand, incorporating dNTPs. RNase H activity degrades the RNA template of the DNA:RNA complex. Thus, reverse transcriptases comprise (a) a binding activity that recognizes and binds to a RNA/DNA hybrid, (b) an RNA-dependent DNA polymerase activity, and (c) an RNase H activity. In addition, reverse transcriptases generally are regarded as having various attributes, including their thermostability, processivity (rate of dNTP incorporation), and fidelity (or error-rate). The reverse transcriptase variants contemplated herein may include any mutations to reverse transcriptase that impacts or changes any one or more of these enzymatic activities (e.g., RNA-dependent DNA polymerase activity, RNase H activity, or

DNA/RNA hybrid-binding activity) or enzyme properties (e.g., thermostability, processivity, or fidelity). Such variants may be available in the art in the public domain, available commercially, or may be made using known methods of mutagenesis, including directed evolutionary processes (e.g., PACE or PANCE).

[0437] In various embodiments, the reverse transcriptase may be a variant reverse transcriptase. As used herein, a "variant reverse transcriptase" includes any naturally occurring or genetically engineered variant comprising one or more mutations (including singular mutations, inversions, deletions, insertions, and rearrangements) relative to a reference sequences (e.g., a reference wild type sequence). RT naturally have several activities, including an RNA-dependent DNA polymerase activity, ribonuclease H activity, and DNA-dependent DNA polymerase activity. Collectively, these activities enable the enzyme to convert single-stranded RNA into double-stranded cDNA. In retroviruses and retrotransposons, this cDNA can then integrate into the host genome, from which new RNA copies can be made via host-cell transcription. Variant RT's may comprise a mutation which impacts one or more of these activities (either which reduces or increases these activities, or which eliminates these activities all together). In addition, variant RTs may comprise one or more mutations which render the RT more or less stable, less prone to aggregration, and facilitates purification and/or detection, and/or other the modification of properties or characteristics.

[0438] A person of ordinary skill in the art will recognize that variant reverse transcriptases derived from other reverse transcriptases, including but not limited to Moloney Murine Leukemia Virus (M-MLV); Human Immunodeficiency Virus (HIV) reverse transcriptase and avian Sarcoma-Leukosis Virus (ASLV) reverse transcriptase, which includes but is not limited to Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Avian Erythroblastosis Virus (AEV) Helper Virus MCAV reverse transcriptase, Avian Myelocytomatosis Virus MC29 Helper Virus MCAV reverse transcriptase, Avian Reticuloendotheliosis Virus (REV-T) Helper Virus REV-A reverse transcriptase, Avian Sarcoma Virus UR2 Helper Virus UR2AV reverse transcriptase, Avian Sarcoma Virus Y73 Helper Virus YAV reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, and Myeloblastosis Associated Virus (MAV) reverse transcriptase may be suitably used in the subject methods and composition described herein.

[0439] One method of preparing variant RTs is by genetic modification (e.g., by modifying the DNA sequence of a wild-type reverse transcriptase). A number of methods are known in

the art that permit the random as well as targeted mutation of DNA sequences (see for example, Ausubel et. al. *Short Protocols in Molecular Biology* (1995) 3.sup.rd Ed. John Wiley & Sons, Inc.). In addition, there are a number of commercially available kits for site-directed mutagenesis, including both conventional and PCR-based methods. Examples include the QuikChange Site-Directed Mutagenesis Kits (AGILENT®), the Q5® Site-Directed Mutagenesis Kit (NEW ENGLAND BIOLABS®), and GeneArt™ Site-Directed Mutagenesis System (THERMOFISHER SCIENTIFIC®).

[0440] In addition, mutant reverse transcriptases may be generated by insertional mutation or truncation (N-terminal, internal, or C-terminal insertions or truncations) according to methodologies known to one skilled in the art. The term "mutation," as used herein, refers to a substitution of a residue within a sequence, e.g., a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). Mutations can include a variety of categories, such as single base polymorphisms, microduplication regions, indel, and inversions, and is not meant to be limiting in any way. Mutations can include "loss-of-function" mutations which is the normal result of a mutation that reduces or abolishes a protein activity. Most loss-of-function mutations are recessive, because in a heterozygote the second chromosome copy carries an unmutated version of the gene coding for a fully functional protein whose presence compensates for the effect of the mutation. Mutations also embrace "gain-of-function" mutations, which is one which confers an abnormal activity on a protein or cell that is otherwise not present in a normal condition. Many gain-of-function mutations are in regulatory sequences rather than in coding regions, and can therefore have a number of consequences. For example, a mutation might lead to one or more genes being expressed in the wrong tissues, these tissues gaining functions that they normally lack. Because of their nature, gain-of-function mutations are usually dominant.

[0441] Older methods of site-directed mutagenesis known in the art rely on sub-cloning of the sequence to be mutated into a vector, such as an M13 bacteriophage vector, that allows the isolation of single-stranded DNA template. In these methods, one anneals a mutagenic

primer (i.e., a primer capable of annealing to the site to be mutated but bearing one or more mismatched nucleotides at the site to be mutated) to the single-stranded template and then polymerizes the complement of the template starting from the 3′ end of the mutagenic primer. The resulting duplexes are then transformed into host bacteria and plaques are screened for the desired mutation.

[0442] More recently, site-directed mutagenesis has employed PCR methodologies, which have the advantage of not requiring a single-stranded template. In addition, methods have been developed that do not require sub-cloning. Several issues must be considered when PCR-based site-directed mutagenesis is performed. First, in these methods it is desirable to reduce the number of PCR cycles to prevent expansion of undesired mutations introduced by the polymerase. Second, a selection must be employed in order to reduce the number of non-mutated parental molecules persisting in the reaction. Third, an extended-length PCR method is preferred in order to allow the use of a single PCR primer set. And fourth, because of the non-template-dependent terminal extension activity of some thermostable polymerases it is often necessary to incorporate an end-polishing step into the procedure prior to blunt-end ligation of the PCR-generated mutant product.

[0443] Methods of random mutagenesis, which will result in a panel of mutants bearing one or more randomly situated mutations, exist in the art. Such a panel of mutants may then be screened for those exhibiting the desired properties, for example, increased stability, relative to a wild-type reverse transcriptase.

[0444] An example of a method for random mutagenesis is the so-called "error-prone PCR method." As the name implies, the method amplifies a given sequence under conditions in which the DNA polymerase does not support high fidelity incorporation. Although the conditions encouraging error-prone incorporation for different DNA polymerases vary, one skilled in the art may determine such conditions for a given enzyme. A key variable for many DNA polymerases in the fidelity of amplification is, for example, the type and concentration of divalent metal ion in the buffer. The use of manganese ion and/or variation of the magnesium or manganese ion concentration may therefore be applied to influence the error rate of the polymerase.

[0445] In various aspects, the RT of the prime editors may be an "error-prone" reverse transcriptase variant. Error-prone reverse transcriptases that are known and/or available in the art may be used. It will be appreciated that reverse transcriptases naturally do not have any proofreading function; thus the error rate of reverse transcriptase is generally higher than

DNA polymerases comprising a proofreading activity. The error-rate of any particular reverse transcriptase is a property of the enzyme's "fidelity," which represents the accuracy of template-directed polymerization of DNA against its RNA template. An RT with high fidelity has a low-error rate. Conversely, an RT with low fidelity has a high-error rate. The fidelity of M-MLV-based reverse transcriptases are reported to have an error rate in the range of one error in 15,000 to 27,000 nucleotides synthesized. See Boutabout et al., "DNA synthesis fidelity by the reverse transcriptase of the yeast retrotransposon Ty1," *Nucleic Acids Res*, 2001, 29: 2217-2222, which is incorporated by reference. Thus, for purposes of this application, those reverse transcriptases considered to be "error-prone" or which are considered to have an "error-prone fidelity" are those having an error rate that is less than one error in 15,000 nucleotides synthesized.

[0446] Error-prone reverse transcriptase also may be created through mutagenesis of a starting RT enzyme (e.g., a wild type M-MLV RT). The method of mutagenesis is not limited and may include directed evolution processes, such as phage-assisted continuous evolution (PACE) or phage-assisted noncontinuous evolution (PANCE). The term "phage-assisted continuous evolution (PACE)," as used herein, refers to continuous evolution that employs phage as viral vectors. The general concept of PACE technology has been described, for example, in International PCT Application, PCT/US2009/056194, filed September 8, 2009, published as WO 2010/028347 on March 11, 2010; International PCT Application, PCT/US2011/066747, filed December 22, 2011, published as WO 2012/088381 on June 28, 2012; U.S. Application, U.S. Patent No. 9,023,594, issued May 5, 2015, International PCT Application, PCT/US2015/012022, filed January 20, 2015, published as WO 2015/134121 on September 11, 2015, and International PCT Application, PCT/US2016/027795, filed April 15, 2016, published as WO 2016/168631 on October 20, 2016, the entire contents of each of which are incorporated herein by reference.

[0447] Error-prone reverse transcriptases may also be obtain by phage-assisted non-continuous evolution (PANCE)," which as used herein, refers to non-continuous evolution that employs phage as viral vectors. PANCE is a simplified technique for rapid in vivo directed evolution using serial flask transfers of evolving 'selection phage' (SP), which contain a gene of interest to be evolved, across fresh *E. coli* host cells, thereby allowing genes inside the host *E. coli* to be held constant while genes contained in the SP continuously evolve. Serial flask transfers have long served as a widely-accessible approach for laboratory evolution of microbes, and, more recently, analogous approaches have been developed for

bacteriophage evolution. The PANCE system features lower stringency than the PACE system.

[0448] Other error-prone reverse transcriptases have been described in the literature, each of which are contemplated for use in the herein methods and compositions. For example, error-prone reverse transcriptases have been described in Bebenek et al., "Error-prone Polymerization by HIV-1 Reverse Transcriptase," *J Biol Chem*, 1993, Vol. 268: 10324-10334 and Sebastian-Martin et al., "Transcriptional inaccuracy threshold attenuates differences in RNA-dependent DNA synthesis fidelity between retroviral reverse transcriptases," *Scientific Reports*, 2018, Vol. 8: 627, each of which are incorporated by reference. Still further, reverse transcriptases, including error-prone reverse transcriptases can be obtained from a commercial supplier, including ProtoScript® (II) Reverse Transcriptase, AMV Reverse Transcriptase, WarmStart® Reverse Transcriptase, and M-MuLV Reverse Transcriptase, all from NEW ENGLAND BIOLABS®, or AMV Reverse Transcriptase XL, SMARTScribe Reverse Transcriptase, GPR ultra-pure MMLV Reverse Transcriptase, all from TAKARA BIO USA, INC. (formerly CLONTECH).

[0449] The herein disclosure also contemplates reverse transcriptases having mutations in RNaseH domain. As mentioned above, one of the intrinsic properties of reverse transcriptases is the RNase H activity, which cleaves the RNA template of the RNA:cDNA hybrid concurrently with polymerization. The RNase H activity can be undesirable for synthesis of long cDNAs because the RNA template may be degraded before completion of full-length reverse transcription. The RNase H activity may also lower reverse transcription efficiency, presumably due to its competition with the polymerase activity of the enzyme. Thus, the present disclosure contemplates any reverse transcriptase variants that comprise a modified RNaseH activity.

[0450] The herein disclosure also contemplates reverse transcriptases having mutations in the RNA-dependent DNA polymerase domain. As mentioned above, one of the intrinsic properties of reverse transcriptases is the RNA-dependent DNA polymerase activity, which incorporates the nucleobases into the nascent cDNA strand as coded by the template RNA strand of the RNA:cDNA hybrid. The RNA-dependent DNA polymerase activity can be increased or decreased (i.e., in terms of its rate of incorporation) to either increase or decrease the processivity of the enzyme. Thus, the present disclosure contemplates any reverse transcriptase variants that comprise a modified RNA-dependent DNA polymerase activity

such that the processivity of the enzyme of either increased or decreased relative to an unmodified version.

[0451] Also contemplated herein are reverse transcriptase variants that have altered thermostability characteristics. The ability of a reverse transcriptase to withstand high temperatures is an important aspect of cDNA synthesis. Elevated reaction temperatures help denature RNA with strong secondary structures and/or high GC content, allowing reverse transcriptases to read through the sequence. As a result, reverse transcription at higher temperatures enables full-length cDNA synthesis and higher yields, which can lead to an improved generation of the 3′ flap ssDNA as a result of the prime editing process. Wild type M-MLV reverse transcriptase typically has an optimal temperature in the range of 37-48°C; however, mutations may be introduced that allow for the reverse transcription activity at higher temperatures of over 48°C, including 49°C, 50°C, 51°C, 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, and higher.

[0452] The variant reverse transcriptases contemplated herein, including error-prone RTs, thermostable RTs, increase-processivity RTs, can be engineered by various routine strategies, including mutagenesis or evolutionary processes. In some cases, the variants can be produced by introducing a single mutation. In other cases, the variants may require more than one mutation. For those mutants comprising more than one mutation, the effect of a given mutation may be evaluated by introduction of the identified mutation to the wild-type gene by site-directed mutagenesis in isolation from the other mutations borne by the particular mutant. Screening assays of the single mutant thus produced will then allow the determination of the effect of that mutation alone.

[0453] Variant RT enzymes used herein may also include other "RT variants" having at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference RT protein, including any wild type RT, or mutant RT, or fragment RT, or other variant of RT disclosed or contemplated herein or known in the art.

[0454] In some embodiments, an RT variant may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or up to 100, or up to 200, or up to 300, or up to 400, or up to 500 or more amino acid changes compared to a reference RT. In some embodiments, the RT variant comprises a fragment of a reference RT, such that the fragment

is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of the reference RT. In some embodiments, the fragment is is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild type RT (M-MLV reverse transcriptase) (*e.g.*, SEQ ID NO: 89) or to any of the reverse transcriptases of SEQ ID NOs: 90-100.

[0455] In some embodiments, the disclosure also may utilize RT fragments which retain their functionality and which are fragments of any herein disclosed RT proteins. In some embodiments, the RT fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, or up to 600 or more amino acids in length.

[0456] In still other embodiments, the disclosure also may utilize RT variants which are truncated at the N-terminus or the C-terminus, or both, by a certain number of amino acids which results in a truncated variant which still retains sufficient polymerase function. In some embodiments, the RT truncated variant has a truncation of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 amino acids at the N-terminal end of the protein. In other embodiments, the RT truncated variant has a truncation of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 amino acids at the C-terminal end of the protein. In still other embodiments, the RT truncated variant has a trunction at the N-terminal and the C-terminal end which are the same or different lengths.

[0457] For example, the prime editors disclosed herein may include a truncated version of M-MLV reverse transcriptase. In this embodiment, the reverse transcriptase contains 4

mutations (D200N, T306K, W313F, T330P; noting that the L603W mutation present in PE2 is no longer present due to the truncation). The DNA sequence encoding this truncated editor is 522 bp smaller than PE2, and therefore makes its potentially useful for applications where delivery of the DNA sequence is challenging due to its size (i.e., adeno-associated virus and lentivirus delivery). This embodiment is referred to as MMLV-RT(trunc) and has the following amino acid sequence:

MMLV-RT(TRUNC)	*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQ APLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSP WNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGL PPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT WTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAA TSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEG QRWLTEARKETVMGQPTPKTPRQLREFLGKAGFCRLFIPGFAEMA APLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFE LFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLR MVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNAR MTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDNSRLI N* (SEQ ID NO: 766)

[0458] In various embodiments, the prime editors disclosed herein may comprise one of the RT variants described herein, or a RT variant thereof having at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any reference Cas9 variants.

[0459] In still other embodiments, the present methods and compositions may utilize a DNA polymerase that has been evolved into a reverse transcriptase, as described in Effefson et al., "Synthetic evolutionary origin of a proofreading reverse transcriptase," *Science*, June 24, 2016, Vol. 352: 1590-1593, the contents of which are incorporated herein by reference.

[0460] In certain other embodiments, the reverse transcriptase is provided as a component of a fusion protein also comprising a napDNAbp. In other words, in some embodiments, the reverse transcriptase is fused to a napDNAbp as a fusion protein.

[0461] In various embodiments, variant reverse transcriptases can be engineered from wild type M-MLV reverse transcriptase as represented by SEQ ID NO: 89.

[0462] In various embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising one or more of the following mutations: P51L, S67K, E69K, L139P, T197A, D200N, H204R, F209N, E302K, E302R, T306K, F309N, W313F, T330P, L345G, L435G, N454K, D524G, E562Q, D583N,

H594Q, L603W, E607K, or D653N in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence.

[0463] Some exemplary reverse transcriptases that can be fused to napDNAbp proteins or provided as individual proteins according to various embodiments of this disclosure are provided below. Exemplary reverse transcriptases include variants with at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to the following wild-type enzymes or partial enzymes:

Description	Sequence (variant substitutions relative to wild type)
Reverse transcriptase (M-MLV RT) wild type moloney murine leukemia virus Used in PE1 (prime editor 1 fusion protein disclosed herein)	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLK ALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLL IENSSP (SEQ ID NO: 89)
M-MLV RT D200N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLK

Description	Sequence (variant substitutions relative to wild type)
	ALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLL IENSSP (SEQ ID NO: 701)
M-MLV RT D200N T330P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLK ALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLL IENSSP (SEQ ID NO: 702)
M-MLV RT D200N T330P L603W	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 740)
M-MLV RT D200N T330P L603W E69K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQ**K**ARLGIKPHIQRLLDQGILVPCQSPWNTP LLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQW YTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRA LLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETV MGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNW GPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQ KLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMG QPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVV ALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTD GSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALK MAEGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILA LLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDT STLLIENSSP (SEQ ID NO: 703)

Description	Sequence (variant substitutions relative to wild type)
M-MLV RT D200N T330P L603W E302R	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLR**R**FLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP(SEQ ID NO: 704)
M-MLV RT D200N T330P L603W E607K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTS**K**GKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 705)
M-MLV RT D200N T330P L603W L139P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSG**P**PPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 706)
M-MLV RT D200N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL

Description	Sequence (variant substitutions relative to wild type)
T330P L603W L435G	LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVI**G**APHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 707)
M-MLV RT D200N T330P L603W N454K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 708)
M-MLV RT D200N T330P L603W T306K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLG**K**AGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 709)
M-MLV RT D200N T330P L603W	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN

Description	Sequence (variant substitutions relative to wild type)
W313F	SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGP DQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 710)
M-MLV RT D200N T330P L603W D524G E562Q D583N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT**G**GS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRA**Q**LIALTQALKMA EGKKLNVYT**N**SRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 711)
M-MLV RT D200N T330P L603W E302R W313F	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLR**R**FLGTAGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 712)
M-MLV RT D200N T330P L603W E607K L139P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSG**P**PPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM

Description	Sequence (variant substitutions relative to wild type)
	GQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTS**K**GKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 713)
M-MLV RT P51L S67K T197A H204R E302K F309N W313F T330P L435G N454K D524G D583N H594Q D653N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LII**L**LKATSTPVSIKQYPM**K**QEARLGIKPHIQRLLDQGILVPCQSPWNTP LLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQW YTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSP**A**LFDEAL**R**RDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTR ALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET VMGQPTPKTPRQLR**K**FLGTAG**N**CRL**F**IPGFAEMAAPLYPLTK**P**GTLFN WGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLT QKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTM GQPLVI**G**APHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPV VALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT **G**GSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALK MAEGKKLNVYT**N**SRYAFATAHI**Q**GEIYRRRGLLTSEGKEIKNKDEILA LLKALFLPKRLSIIHCPGHQKGHSAEARGNRMA**N**QAARKAAITETPDT STLLIENSSP (SEQ ID NO: 714)
M-MLV RT D200N P51L S67K T197A H204R E302K F309N W313F T330P L345G N454K D524G D583N H594Q D653N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LII**L**LKATSTPVSIKQYPM**K**QEARLGIKPHIQRLLDQGILVPCQSPWNTP LLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQW YTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSP**A**LF**N**EAL**R**RDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTR ALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET VMGQPTPKTPRQLR**K**FLGTAG**N**CRL**F**IPGFAEMAAPLYPLTK**P**GTLFN WGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLT QKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTM GQPLVI**G**APHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPV VALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT **G**GSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALK MAEGKKLNVYT**N**SRYAFATAHI**Q**GEIYRRRGLLTSEGKEIKNKDEILA LLKALFLPKRLSIIHCPGHQKGHSAEARGNRMA**N**QAARKAAITETPDT STLLIENSSP (SEQ ID NO: 715)
M-MLV RT D200N T330P L603W T306K W313F in PE2	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAP LIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLREFLG**K**AGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKL

Description	Sequence (variant substitutions relative to wild type)
	GPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQP LVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVAL NPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGS SLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMA EGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALL KALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTST LLIENSSP (SEQ ID NO: 716)

[0464] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising one or more of the following mutations: P51X, S67X, E69X, L139X, T197X, D200X, H204X, F209X, E302X, T306X, F309X, W313X, T330X, L345X, L435X, N454X, D524X, E562X, D583X, H594X, L603X, E607X, or D653X in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid.

[0465] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a P51X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is L.

[0466] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a S67X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0467] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a E69X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0468] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a L139X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is P.

[0469] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a T197X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is A.

[0470] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a D200X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is N.

[0471] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a H204X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is R.

[0472] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a F209X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is N.

[0473] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a E302X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0474] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a E302X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is R.

[0475] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a T306X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in

another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0476] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a F309X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is N.

[0477] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a W313X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is F.

[0478] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a T330X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is P.

[0479] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a L345X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is G.

[0480] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a L435X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is G.

[0481] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a N454X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0482] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a D524X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is G.

[0483] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a E562X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is Q.

[0484] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a D583X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is N.

[0485] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a H594X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is Q.

[0486] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a L603X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is W.

[0487] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a E607X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is K.

[0488] In various other embodiments, the prime editors described herein (with RT provided as either a fusion partner or *in trans*) can include a variant RT comprising a D653X mutation in the wild type M-MLV RT of SEQ ID NO: 89 or at a corresponding amino acid position in

another wild type RT polypeptide sequence, wherein "X" can be any amino acid. In certain embodiments, X is N.

[0489] Some exemplary reverse transcriptases that can be fused to napDNAbp proteins or provided as individual proteins according to various embodiments of this disclosure are provided below. Exemplary reverse transcriptases include variants with at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to the following wild-type enzymes or partial enzymes:

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
REVERSE TRANSCRIPTASE (M-MLV RT) WILD TYPE MOLONEY MURINE LEUKEMIA VIRUS USED IN PE1 (PRIME EDITOR 1 FUSION PROTEIN DISCLOSED HEREIN)	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 89)
M-MLV RT D200N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 106)
M-MLV RT D200N T330P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 107)
M-MLV RT D200N T330P L603W	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 108)
M-MLV RT E69K D200N T330P L603W	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQ**K**ARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 109)
M-MLV RT D200N T330P L603W E302R	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLR**R**FLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP(S EQ ID NO: 110)
M-MLV RT D200N T330P L603W E607K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTS**K**GKEIKNKDEILALLKALFLPK RLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 111)
M-MLV RT D200N T330P L603W L139P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSG**P**PPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 112)
M-MLV RT D200N T330P L603W L435G	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI**G**A PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 113)
M-MLV RT D200N T330P L603W N454K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 114)
M-MLV RT D200N T330P L603W T306K	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLG**K**AGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPD QQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGP WRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVIL APHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPAT LLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQ EGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLP KRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSS P (SEQ ID NO: 115)
M-MLV RT D200N T330P L603W W313F	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 116)
M-MLV RT D200N T330P L603W D524G E562Q D583N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT**G**GSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRA**Q**LIALTQALKMAEGKKLN VYT**N**SRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 117)
M-MLV RT D200N T330P L603W E302R W313F	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLR**R**FLGTAGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 118)
M-MLV RT D200N T330P L603W E607K L139P	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSG**P**PPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTS**K**GKEIKNKDEILALLKALFLPK RLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 119)
M-MLV RT P51L S67K T197A H204R E302K F309N W313F T330P L435G N454K D524G D583N H594Q D653N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL II**L**LKATSTPVSIKQYPM**K**QEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SP**A**LFDEAL**R**RDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLR**K**FLGTAG**N**CRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGP DQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI **G**APHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPVVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT**G**GSSLL QEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGK KLNVYT**N**SRYAFATAHI**Q**GEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMA**N**QAARKAAITETPDTSTLLIENS SP (SEQ ID NO: 120)
M-MLV RT P51L S67K T197A D200N H204R E302K F309N W313F T330P L345G N454K D524G D583N H594Q D653N	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL II**L**LKATSTPVSIKQYPM**K**QEARLGIKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWY TVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN SP**A**LF**N**EAL**R**RDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVM GQPTPKTPRQLR**K**FLGTAG**N**CRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGP DQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI **G**APHAVEALVKQPPDRWLS**K**ARMTHYQALLLDTDRVQFGPVVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYT**G**GSSLL QEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGK KLNVYT**N**SRYAFATAHI**Q**GEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMA**N**QAARKAAITETPDTSTLLIENS SP (SEQ ID NO: 121)
M-MLV RT D200N T330P L603W T306K W313F IN PE2	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLG**K**AGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR

DESCRIPTION	SEQUENCE (VARIANT SUBSTITUTIONS RELATIVE TO WILD TYPE)
	LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 122)

[0490] The prime editor (PE) system described here contemplates any publicly-available reverse transcriptase described or disclosed in any of the following U.S. patents (each of which are incorporated by reference in their entireties): U.S. Patent Nos: 10,202,658; 10,189,831; 10,150,955; 9,932,567; 9,783,791; 9,580,698; 9,534,201; and 9,458,484, and any variant thereof that can be made using known methods for installing mutations, or known methods for evolving proteins. The following references describe reverse transcriptases in art. Each of their disclosures are incorporated herein by reference in their entireties.

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[0522] Any of the references noted above which relate to reverse transriptases are hereby incorporated by reference in their entireties, if not already stated so.

[4] PE fusion proteins

[0523] The prime editor (PE) system described herein contemplate fusion proteins comprising a napDNAbp and a polymerase (e.g., DNA-dependent DNA polymerase or RNA-dependent DNA polymerase, such as, reverse transcriptase), and optionally joined by a linker. The application contemplates any suitable napDNAbp and polymerase (e.g., DNA-dependent DNA polymerase or RNA-dependent DNA polymerase, such as, reverse transcriptase) to be combined in a single fusion protein. Examples of napDNAbps and polymerases (e.g., DNA-dependent DNA polymerase or RNA-dependent DNA polymerase, such as, reverse transcriptase) are each defined herein. Since polymerases are well-known in the art, and the

amino acid sequences are readily available, this disclosure is not meant in any way to be limited to those specific polymerases identified herein.

[0524] In various embodiments, the fusion proteins may comprise any suitable structural configuration. For example, the fusion protein may comprise from the N-terminus to the C-terminus direction, a napDNAbp fused to a polymerase (e.g., DNA-dependent DNA polymerase or RNA-dependent DNA polymerase, such as, reverse transcriptase) . In other embodiments, the fusion protein may comprise from the N-terminus to the C-terminus direction, a polymerase (e.g., a reverse transcriptase) fused to a napDNAbp. The fused domain may optionally be joined by a linker, e.g., an amino acid sequence. In other embodiments, the fusion proteins may comprise the structure NH_2-[napDNAbp]-[polymerase]-COOH; or NH_2-[polymerase]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence. In embodiments wherein the polymerase is a reverse transcriptase, the fusion proteins may comprise the structure NH_2-[napDNAbp]-[RT]-COOH; or NH_2-[RT]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

[0525] An exemplary fusion protein is depicted in FIG. 14, which shows a fusion protein comprising an MLV reverse transcriptase ("MLV-RT") fused to a nickase Cas9 ("Cas9(H840A)") via a linker sequence. This example is not intended to limit scope of fusion proteins that may be utilized for the prime editor (PE) system described herein.

[0526] In various embodiments, the prime editor fusion protein may have the following amino acid sequence (referred to herein as "PE1"), which includes a Cas9 variant comprising an H840A mutation (i.e., a Cas9 nickase) and an M-MLV RT wild type, as well as an N-terminal NLS sequence (19 amino acids) and an amino acid linker (32 amino acids) that joins the C-terminus of the Cas9 nickase domain to the N-terminus of the RT domain. The PE1 fusion protein has the following structure: [NLS]-[Cas9(H840A)]-[linker]-[MMLV_RT(wt)]. The amino acid sequence of PE1 and its individual components are as follows:

DESCRIPTION	SEQUENCE
PE1 FUSION PROTEIN CAS9(H840A)-MMLV_RT(WT)	MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPS KKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNI VDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF LIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDA KLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRV NTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ**

DESCRIPTION	SEQUENCE
	SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIE RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHE HIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYL YYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTR SDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKV LSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDE IIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS QLGGDSGGSSGGSSGSETPGTSESATPESSGGSSGGSS*TLNIEDEYRLH ETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQ YPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQD LREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQ PLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHP DLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQV KYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREFLGTAGFCRLWIPG FAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFE LFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAV LTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTD RVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADH TWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALK MAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKAL FLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSS PSGGSKRTADGSEFEPKKKRKV* (SEQ ID NO: 123) KEY: NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133) **CAS9(H840A) (SEQ ID NO: 126) **33-AMINO ACID LINKER** (SEQ ID NO: 127) *M-MLV REVERSE TRANSCRIPTASE* (SEQ ID NO: 128)
PE1 – N-TERMINAL NLS	MKRTADGSEFESPKKKRKV (SEQ ID NO: 124)

DESCRIPTION	SEQUENCE
PE1 – CAS9 (H840A) (MET MINUS)	DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFH RLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDK ADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLG LTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKIL TFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIE RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNA SLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSD GFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKK GILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN RLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA RKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG GD (SEQ ID NO: 130)
PE1 – LINKER BETWEEN CAS9 DOMAIN AND RT DOMAIN (33 AMINO ACIDS)	SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127)
PE1 – M-MLV RT	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA

DESCRIPTION	SEQUENCE
	PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 132)
PE1 – C-TERMINAL NLS	SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 133)

[0527] In another embodiment, the prime editor fusion protein may have the following amino acid sequence (referred to herein as "PE2"), which includes a Cas9 variant comprising an H840A mutation (i.e., a Cas9 nickase) and an M-MLV RT comprising mutations D200N, T330P, L603W, T306K, and W313F, as well as an N-terminal NLS sequence (19 amino acids) and an amino acid linker (33 amino acids) that joins the C-terminus of the Cas9 nickase domain to the N-terminus of the RT domain. The PE2 fusion protein has the following structure: [NLS]-[Cas9(H840A)]-[linker]-[MMLV_RT(D200N)(T330P)(L603W)(T306K)(W313F)]. The amino acid sequence of PE2 is as follows:

| PE2 FUSION PROTEIN

CAS9(H840A)-MMLV_RT D200N T330P L603W T306K W313F | MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPS KKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNI VDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF LIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDA KLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRV NTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIE RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHE HIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYL YYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTR SDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKV**

	LSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDE IIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS QLGGDSGGSSGGSSGSETPGTSESATPESSGGSSGGSS*TLNIEDEYRLH ETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQ YPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQD LREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQ PLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHP DLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQV KYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREFLGKAGFCRLFIPG FAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFE LFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAV LTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTD RVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADH TWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALK MAEGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKA LFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENS SP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 134)

KEY:
NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 137)
33-AMINO ACID LINKER (SEQ ID NO: 127)
M-MLV REVERSE TRANSCRIPTASE (SEQ ID NO: 139) |
| PE2 – N-TERMINAL NLS | MKRTADGSEFESPKKKRKV (SEQ ID NO: 124) |
| PE2 – CAS9 (H840A) (MET MINUS) | DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFH RLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDK ADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLG LTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPE KYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLN REDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKIL TFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIE RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNA SLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTY AHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSD GFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKK GILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN RLSDYDVD**A**IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKM |

	KNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQI TKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMI AKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA RKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIM ERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG GD (SEQ ID NO: 141)
PE2 – LINKER BETWEEN CAS9 DOMAIN AND RT DOMAIN (33 AMINO ACIDS)	SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127)
PE2 – MMLV_RT D200N T330P L603W T306K W313F	TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPL IIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYT VLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSP TLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQ TLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQ PTPKTPRQLREFLG**K**AGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILA PHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEG QRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKR LSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 143)
PE2 – C-TERMINAL NLS	SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 133)

[0528] In still other embodiments, the prime editor fusion protein may have the following amino acid sequences:

PE FUSION PROTEIN MMLV_RT(WT)-32AA-CAS9(H840A)	MKRTADGSEFESPKKKRKV*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQ AWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLD QGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYN LLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT WTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELD CQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARK ETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNW GPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGP*

	*WRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPH AVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEE GLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAA VTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH IHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAE ARGNRMADQAARKAAITETPDTSTLLIENSSP**<u>SGGSSGGSSGSETPGTSES ATPESSGGSSGGSS</u>**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKV LGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRIC YLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGD LNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSR RLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQL SKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEIT KAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRT FDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYV GPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSG EQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEER LKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTIL DFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIAN LAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ KGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL QNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDK NRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERG GLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIRE VKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALI KKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIM NFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSM PQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNEL ALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQ ISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLG APAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG GD**<u>SGGSKRTADGSEFEPKKKRKV</u>** (SEQ ID NO: 145) KEY: <u>NUCLEAR LOCALIZATION SEQUENCE (NLS)</u> TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133) **CAS9(H840A)** (SEQ ID NO: 147) **<u>33-AMINO ACID LINKER</u>** (SEQ ID NO: 127) *M-MLV REVERSE TRANSCRIPTASE* (SEQ ID NO: 149)
PE FUSION PROTEIN MMLV_RT(WT)-60AA-	<u>MKRTADGSEFESPKKKRKV</u>*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQ AWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLD QGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYN LLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT WTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELD*

CAS9(H840A)	*CQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARK ETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNW GPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGP WRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPH AVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEE GLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAA VTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAH IHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAE ARGNRMADQAARKAAITETPDTSTLLIENSSPS*<u>**GGSSGGSSGSETPGTSES ATPESAGSYPYDVPDYAGSAAPAAKKKKLDGSGSGGSSGGS**</u>**DKKYS IGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFH RLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDST DKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLF GNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGD QYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQD LTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIK PILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKS EETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVT VKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFL DNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKE LGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSD YDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKN YWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQ ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYD VRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIE TNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKES ILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSK KLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYS LFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKE VLDATLIHQSITGLYETRIDLSQLGGD*<u>SGGSKRTADGSEFEPKKKRKV</u> (SEQ ID NO: 150) KEY: <u>NUCLEAR LOCALIZATION SEQUENCE (NLS)</u> TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133) **CAS9(H840A)**(SEQ ID NO: 153) **<u>AMINO ACID LINKER</u>** *M-MLV REVERSE TRANSCRIPTASE*

PE FUSION PROTEIN CAS9(H840A)-FEN1-MMLV_RT D200N T330P L603W T306K W313F	MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPS KKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNI VDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHF LIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSAR LSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDA KLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRV NTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFR IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIE RMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPA FLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHE HIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYL YYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTR SDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYS NIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKV LSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKY GGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNP IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKG NELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDE IIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLT NLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLS QLGGD**<u>SGGSSGGSSGSETPGTSESATPESSGGSSGGSS</u>**GIQGLAKLIA DVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQGGDVLQNEEGETT SHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELAKRSERRAEAE KQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGIPYLDA PSEAEASCAALVKAGKVYAAATEDMDCLTFGSPVLMRHLTASEAKKLP IQEFHLSRILQELGLNQEQFVDLCILLGSDYCESIRGIGPKRAVDLIQKHK SIEEIVRRLDPNKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEPNE EELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLDDFFKVTGSLSS AKRKEPEPKGSTKKKAKTGAAGKFKRGK**<u>SGGSSGGSSGSETPGTSES ATPESSGGSSGGSS</u>***TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETG GMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPC QSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPP SHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQG FKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRA LLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQP TPKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQK AYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVA YLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALV KQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHN**

	*CLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTET EVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEI YRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGN RMADQAARKAAITETPDTSTLLIENSSP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 154) KEY: NUCLEAR LOCALIZATION SEQUENCE (NLS) TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133) **CAS9(H840A)** (SEQ ID NO: 157) **33-AMINO ACID LINKER 1** (SEQ ID NO: 127) *M-MLV REVERSE TRANSCRIPTASE* (SEQ ID NO: 159) **33-AMINO ACID LINKER 2** (SEQ ID NO: 127) FEN1 (SEQ ID NO: 161)

[0529] In other embodiments, the prime editor fusion proteins can be based on SaCas9 or on SpCas9 nickases with altered PAM specificities, such as the following exemplary sequences:

SACAS9-M-MLV RT PRIME EDITOR	MKRTADGSEFESPKKKRKVGKRNYILGLDIGITSVGYGIIDYETR DVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVKK LLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLA KRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLE RLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFID TYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPE ELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYH DIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQE EIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKL VPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYG LPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGK ENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII PRSVSFDNSFNNKVLVKQEEASKKGNRTPFQYLSSSDSKISYETF KKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVD TRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFK KERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMF EEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKK PNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLIN KSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGN YLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVK LSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYE EAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNL YEVKSKKHPQIIKKGSGGSSGGSSGSETPGTSESATPESSGGSSG GSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGL AVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGIL VPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVP NPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR DPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDL ILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQ ICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF

	LGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEI KQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRR PVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI LAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVV ALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHT WYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAEL IALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSE GKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRM ADQAARKAAITETPDTSTLLIENSSPSGGSKRTADGSEFEPKKKR KV (SEQ ID NO: 162)
SPCAS9(H840A)-VRQR-MALONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE PRIME EDITOR	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT

	PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 163)
SPCAS9(H840A)-VRER-MALONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE PRIME EDITOR	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKEYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT

	PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 164)

[0530] In yet other embodiments, the prime editor fusion proteins contemplated herein may include a Cas9 nickase (e.g., Cas9 (H840A)) fused to a truncated version of M-MLV reverse transcriptase. In this embodiment, the reverse transcriptase also contains 4 mutations (D200N, T306K, W313F, T330P; noting that the L603W mutation present in PE2 is no longer present due to the truncation). The DNA sequence encoding this truncated editor is 522 bp smaller than PE2, and therefore makes its potentially useful for applications where delivery of the DNA sequence is challenging due to its size (i.e. adeno-associated virus and lentivirus delivery). This embodiment is referred to as Cas9(H840A)-MMLV-RT(trunc) or "PE2-short"or "PE2-trunc" and has the following amino acid sequence:

CAS9(H840A)-MMLV-RT(TRUNC) OR PE2-SHORT	MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDE** **YKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLK** **RTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLV** **EEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDK** **ADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ** **TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGE** **KKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDD** **DLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKA** **PLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK** **NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNRED** **LLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREK** **IEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEV** **VDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN** **ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVK** **QLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDK** **DFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDK** **VMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSD** **GFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL** **AGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ** **TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNE** **KLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDD** **SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK** **LITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV** **AQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF** **YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDY**

	KVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLAN GEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIV KKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDS PTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKN PIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFV EQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKP IREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQSITGLYETRIDLSQLGGD<u>**SGGSSGGSSGSETPGTSE SATPESSGGSSGGSS**</u>*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQ AWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHI QRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVE DIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFA FEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQH PDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQI CQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREFLGK AGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQAL LTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSK KLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEA LVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLP EEGLQHNCLDNSRLIN*<u>SGGSKRTADGSEFEPKKKRKV</u> (SEQ ID NO: 765)

KEY:
<u>NUCLEAR LOCALIZATION SEQUENCE (NLS)</u> TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 157)
<u>33-AMINO ACID LINKER 1</u> (SEQ ID NO: 127)
M-MLV TRUNCATED REVERSE TRANSCRIPTASE (SEQ ID NO: 766) |

[0531] See FIG. 75, which provides a bar graph comparing the efficiency (i.e., "% of total sequencing reads with the specified eidt or indels") of PE2, PE2-trunc, PE3, and PE3-trunc over different target sites in various cell lines. The data shows that the prime editors comprising the truncated RT variants were about as efficient as the prime editors comprising the non-trunctated RT proteins.

[0532] In various embodiments, the prime editor fusion proteins contemplated herein may also include any variants of the above-disclosed sequences having an amino acid sequence that is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to PE1, PE2, or any of the above indicated prime editor fusion sequences.

[0533] In certain embodiments, linkers may be used to link any of the peptides or peptide domains or moieties of the invention (e.g., a napDNAbp linked or fused to a reverse transcriptase).

[5] Linkers and other domains

[0534] The PE fusion proteins may comprise various other domains besides the napDNAbp (e.g., Cas9 domain) and the polymerase domain (e.g., RT domain). For example, in the case where the napDNAbp is a Cas9 and the polymerase is a RT, the PE fusion proteins may comprise one or more linkers that join the Cas9 domain with the RT domain. The linkers may also join other functional domains, such as nuclear localization sequences (NLS) or a FEN1 (or other flap endonuclease) to the PE fusion proteins or a domain thereof.

[0535] In addition, in embodiments involving *trans* prime editing, linkers may be used to link tPERT recruitment protein to a prime editor, e.g., between the tPERt recruitment protein and the napDNAbp. See e.g., FIG. 3G for an exemplary schematic of a *trans* prime editor (tPE) that includes linkers to separately fuse a polymerase domain and a recruiting protein domain to a napDNAbp.

A. Linkers

[0536] As defined above, the term "linker," as used herein, refers to a chemical group or a molecule linking two molecules or moieties, e.g., a binding domain and a cleavage domain of a nuclease. In some embodiments, a linker joins a gRNA binding domain of an RNA-programmable nuclease and the catalytic domain of a polymerase (e.g., a reverse transcriptase). In some embodiments, a linker joins a dCas9 and reverse transcriptase. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-100 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated.

[0537] The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polpeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (e.g., a carbon-carbon bond, disulfide bond, carbon-heteroatom

bond, etc.). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (e.g., polyethylene, polyethylene glycol, polyamide, polyester, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (e.g., glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminohexanoic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (e.g., cyclopentane, cyclohexane). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the linker is based on a phenyl ring. The linker may included funtionalized moieties to facilitate attachment of a nucleophile (e.g., thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

[0538] In some other embodiments, the linker comprises the amino acid sequence $(GGGGS)_n$ (SEQ ID NO: 165), $(G)_n$ (SEQ ID NO: 166), $(EAAAK)_n$ (SEQ ID NO: 167), $(GGS)_n$ (SEQ ID NO: 168), $(SGGS)_n$ (SEQ ID NO: 169), $(XP)_n$ (SEQ ID NO: 170), or any combination thereof, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, the linker comprises the amino acid sequence (GGS)N (SEQ ID NO: 176), wherein n is 1, 3, or 7. In some embodiments, the linker comprises the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 171). In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 172). In some embodiments, the linker comprises the amino acid sequence SGGSGGSGGS (SEQ ID NO: 173). In some embodiments, the linker comprises the amino acid sequence SGGS (SEQ ID NO: 174). In other embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESAGSYPYDVPDYAGSAAPAAKKKKLDGSGSGGSSGGS (SEQ ID NO: 175, 60AA).

[0539] In certain embodiments, linkers may be used to link any of the peptides or peptide domains or moieties of the invention (e.g., a napDNAbp linked or fused to a reverse transcriptase).

[0540] As defined above, the term "linker," as used herein, refers to a chemical group or a molecule linking two molecules or moieties, e.g., a binding domain and a cleavage domain of a nuclease. In some embodiments, a linker joins a gRNA binding domain of an RNA-programmable nuclease and the catalytic domain of a recombinase. In some embodiments, a linker joins a dCas9 and reverse transcriptase. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 5-100 amino acids in length, for example, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated.

[0541] The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (e.g., a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, etc.). In certain embodiments, the linker is a carbon-nitrogen bond of an amide linkage. In certain embodiments, the linker is a cyclic or acyclic, substituted or unsubstituted, branched or unbranched aliphatic or heteroaliphatic linker. In certain embodiments, the linker is polymeric (e.g., polyethylene, polyethylene glycol, polyamide, polyester, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of aminoalkanoic acid. In certain embodiments, the linker comprises an aminoalkanoic acid (e.g., glycine, ethanoic acid, alanine, beta-alanine, 3-aminopropanoic acid, 4-aminobutanoic acid, 5-pentanoic acid, etc.). In certain embodiments, the linker comprises a monomer, dimer, or polymer of amino*HEXA*noic acid (Ahx). In certain embodiments, the linker is based on a carbocyclic moiety (e.g., cyclopentane, cyclo*HEXA*ne). In other embodiments, the linker comprises a polyethylene glycol moiety (PEG). In other embodiments, the linker comprises amino acids. In certain embodiments, the linker comprises a peptide. In certain embodiments, the linker comprises an aryl or heteroaryl moiety. In certain embodiments, the

linker is based on a phenyl ring. The linker may included funtionalized moieties to facilitate attachment of a nucleophile (e.g., thiol, amino) from the peptide to the linker. Any electrophile may be used as part of the linker. Exemplary electrophiles include, but are not limited to, activated esters, activated amides, Michael acceptors, alkyl halides, aryl halides, acyl halides, and isothiocyanates.

[0542] In some other embodiments, the linker comprises the amino acid sequence (GGGGS)n (SEQ ID NO: 165), (G)n (SEQ ID NO: 166), $(EAAAK)_n$ (SEQ ID NO: 167), $(GGS)_n$ (SEQ ID NO: 168), (SGGS)n (SEQ ID NO: 169), (XP)n (SEQ ID NO: 170), or any combination thereof, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, the linker comprises the amino acid sequence (GGS)N (SEQ ID NO: 176), wherein n is 1, 3, or 7. In some embodiments, the linker comprises the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 171). In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 172). In some embodiments, the linker comprises the amino acid sequence SGGSGGSGGS (SEQ ID NO: 173). In some embodiments, the linker comprises the amino acid sequence SGGS (SEQ ID NO: 174).

[0543] In particular, the following linkers can be used in various embodiments to join prime editor domains with one another:

GGS (SEQ ID NO: 767);

GGSGGS (SEQ ID NO: 768);

GGSGGSGGS (SEQ ID NO: 769);

SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127);

SGSETPGTSESATPES (SEQ ID NO: 171);

SGGSSGGSSGSETPGTSESATPESAGSYPYDVPDYAGSAAPAAKKKKLDGSGSGGSS GG S (SEQ ID NO: 175).

B. Nuclear localization sequence (NLS)

[0544] In various embodiments, the PE fusion proteins may comprise one or more nuclear localization sequences (NLS), which help promote translocation of a protein into the cell nucleus. Such sequences are well-known in the art and can include the following examples:

DESCRIPTION	SEQUENCE	SEQ ID NO:
NLS OF SV40	PKKKRKV	SEQ ID NO: 16

LARGE T-AG		
NLS	MKRTADGSEFESPKKKRKV	SEQ ID NO: 124
NLS	MDSLLMNRRKFLYQFKNVRWAKGRRETYLC	SEQ ID NO: 17
NLS OF NUCLEOPLASMIN	AVKRPAATKKAGQAKKKKLD	SEQ ID NO: 190
NLS OF EGL-13	MSRRRKANPTKLSENAKKLAKEVEN	SEQ ID NO: 191
NLS OF C-MYC	PAAKRVKLD	SEQ ID NO: 192
NLS OF TUS-PROTEIN	KLKIKRPVK	SEQ ID NO: 193
NLS OF POLYOMA LARGE T-AG	VSRKRPRP	SEQ ID NO: 194
NLS OF HEPATITIS D VIRUS ANTIGEN	EGAPPAKRAR	SEQ ID NO: 195
NLS OF MURINE P53	PPQPKKKPLDGE	SEQ ID NO: 196
NLS OF PE1 AND PE2	SGGSKRTADGSEFEPKKKRKV	SEQ ID NO: 133

[0545] The NLS examples above are non-limiting. The PE fusion proteins may comprise any known NLS sequence, including any of those described in Cokol et al., "Finding nuclear localization signals," *EMBO Rep.,* 2000, 1(5): 411-415 and Freitas et al., "Mechanisms and Signals for the Nuclear Import of Proteins," *Current Genomics*, 2009, 10(8): 550-7, each of which are incorporated herein by reference.

[0546] In various embodiments, the prime editors and constructs encoding the prime editors disclosed herein further comprise one or more, preferably, at least two nuclear localization signals. In certain embodiments, the prime editors comprise at least two NLSs. In embodiments with at least two NLSs, the NLSs can be the same NLSs or they can be different NLSs. In addition, the NLSs may be expressed as part of a fusion protein with the remaining portions of the prime editors. In some embodiments, one or more of the NLSs are bipartite NLSs ("bpNLS"). In certain embodiments, the disclosed fusion proteins comprise two bipartite NLSs. In some embodiments, the disclosed fusion proteins comprise more than two bipartite NLSs.

[0547] The location of the NLS fusion can be at the N-terminus, the C-terminus, or within a sequence of a prime editor (e.g., inserted between the encoded napDNAbp component (e.g., Cas9) and a polymerase domain (e.g., a reverse transcriptase domain).

[0548] The NLSs may be any known NLS sequence in the art. The NLSs may also be any future-discovered NLSs for nuclear localization. The NLSs also may be any naturally-occurring NLS, or any non-naturally occurring NLS (e.g., an NLS with one or more desired mutations).

[0549] The term "nuclear localization sequence" or "NLS" refers to an amino acid sequence that promotes import of a protein into the cell nucleus, for example, by nuclear transport. Nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank et al., International PCT application PCT/EP2000/011690, filed November 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference. In some embodiments, an NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 16), MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 17), KRTADGSEFESPKKKRKV (SEQ ID NO: 3864), or KRTADGSEFEPKKKRKV (SEQ ID NO: 13). In other embodiments, NLS comprises the amino acid sequences NLSKRPAAIKKAGQAKKKK (SEQ ID NO: 3865), PAAKRVKLD (SEQ ID NO: 192), RQRRNELKRSF (SEQ ID NO: 3866), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 3867).

[0550] In one aspect of the disclosure, a prime editor may be modified with one or more nuclear localization signals (NLS), preferably at least two NLSs. In certain embodiments, the prime editors are modified with two or more NLSs. The disclosure contemplates the use of any nuclear localization signal known in the art at the time of the disclosure, or any nuclear localization signal that is identified or otherwise made available in the state of the art after the time of the instant filing. A representative nuclear localization signal is a peptide sequence that directs the protein to the nucleus of the cell in which the sequence is expressed. A nuclear localization signal is predominantly basic, can be positioned almost anywhere in a protein's amino acid sequence, generally comprises a short sequence of four amino acids (Autieri & Agrawal, (1998) *J. Biol. Chem.* 273: 14731-37, incorporated herein by reference) to eight amino acids, and is typically rich in lysine and arginine residues (Magin *et al.*, (2000) *Virology* 274: 11-16, incorporated herein by reference). Nuclear localization signals often comprise proline residues. A variety of nuclear localization signals have been identified and

have been used to effect transport of biological molecules from the cytoplasm to the nucleus of a cell. See, e.g., Tinland *et al.*, (1992) *Proc. Natl. Acad. Sci. U.S.A.* 89:7442-46; Moede *et al.*, (1999) *FEBS Lett.* 461:229-34, which is incorporated by reference. Translocation is currently thought to involve nuclear pore proteins.

[0551] Most NLSs can be classified in three general groups: (i) a monopartite NLS exemplified by the SV40 large T antigen NLS (PKKKRKV (SEQ ID NO: 16)); (ii) a bipartite motif consisting of two basic domains separated by a variable number of spacer amino acids and exemplified by the *Xenopus nucleoplasmin* NLS (KRXXXXXXXXXXKKKL (SEQ ID NO: 3868)); and (iii) noncanonical sequences such as M9 of the hnRNP Al protein, the influenza virus nucleoprotein NLS, and the yeast Gal4 protein NLS (Dingwall and Laskey 1991).

[0552] Nuclear localization signals appear at various points in the amino acid sequences of proteins. NLS's have been identified at the N-terminus, the C-terminus and in the central region of proteins. Thus, the disclosure provides prime editors that may be modified with one or more NLSs at the C-terminus, the N-terminus, as well as at in internal region of the prime editor. The residues of a longer sequence that do not function as component NLS residues should be selected so as not to interfere, for example tonically or sterically, with the nuclear localization signal itself. Therefore, although there are no strict limits on the composition of an NLS-comprising sequence, in practice, such a sequence can be functionally limited in length and composition.

[0553] The present disclosure contemplates any suitable means by which to modify a prime editor to include one or more NLSs. In one aspect, the prime editors may be engineered to express a prime editor protein that is translationally fused at its N-terminus or its C-terminus (or both) to one or more NLSs, i.e., to form a prime editor-NLS fusion construct. In other embodiments, the prime editor-encoding nucleotide sequence may be genetically modified to incorporate a reading frame that encodes one or more NLSs in an internal region of the encoded prime editor. In addition, the NLSs may include various amino acid linkers or spacer regions encoded between the prime editor and the N-terminally, C-terminally, or internally-attached NLS amino acid sequence, e.g, and in the central region of proteins. Thus, the present disclosure also provides for nucleotide constructs, vectors, and host cells for expressing fusion proteins that comprise a prime editor and one or more NLSs.

[0554] The prime editors described herein may also comprise nuclear localization signals which are linked to a prime editor through one or more linkers, e.g., and polymeric, amino

acid, nucleic acid, polysaccharide, chemical, or nucleic acid linker element. The linkers within the contemplated scope of the disclosure are not intented to have any limitations and can be any suitable type of molecule (e.g., polymer, amino acid, polysaccharide, nucleic acid, lipid, or any synthetic chemical linker domain) and be joined to the prime editor by any suitable strategy that effectuates forming a bond (e.g., covalent linkage, hydrogen bonding) between the prime editor and the one or more NLSs.

C. Flap endonucleases (e.g., FEN1)

[0555] In various embodiments, the PE fusion proteins may comprise one or more flap endonulceases (e.g., FEN1), which refers to an enzyme that catalyzes the removal of 5′ single strand DNA flaps. These are naturally occurring enzymes that process the removal of 5′ flaps formed during cellular processes, including DNA replication. The prime editing methods herein described may utilize endogenously supplied flap endonucleases or those provided *in trans* to remove the 5′ flap of endogenouse DNA formed at the target site during prime editing. Flap endonucleases are known in the art and can be found described in Patel et al., "Flap endonucleases pass 5′-flaps through a flexible arch using a disorder-thread-order mechanism to confer specificity for free 5′-ends," *Nucleic Acids Research*, 2012, 40(10): 4507-4519 and Tsutakawa et al., "Human flap endonuclease structures, DNA double-base flipping, and a unified understanding of the FEN1 superfamily," *Cell*, 2011, 145(2): 198-211 (each of which are incorporated herein by reference). An exemplary flap endonuclease is FEN1, which can be represented by the following amino acid sequence:

Description	Sequence	SEQ ID NO:
FEN1 Wild type (wt)	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSI YQFLIAVRQGGDVLQNEEGETTSHLMGMFYRTIRMME NGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQ AQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI PYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGS PVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFVD LCILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLDP NKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEPN EEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLD DFFKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFKR GK	SEQ ID NO: 198

[0556] The flap endonucleases may also include any FEN1 variant, mutant, or other flap endonuclease ortholog, homolog, or variant. Non-limiting FEN1 variant examples are as follows:

Description	Sequence	SEQ ID NO:

FEN1 K168R (relative to FEN1 wt)	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSI YQFLIAVRQGGDVLQNEEGETTSHLMGMFYRTIRMME NGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQ AQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI PYLDAPSEAEASCAALV**R**AGKVYAAATEDMDCLTFGS PVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFVD LCILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLDP NKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEPN EEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLD DFFKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFKR GK	SEQ ID NO: 199
FEN1 S187A (relative to FEN1 wt)	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSI YQFLIAVRQGGDVLQNEEGETTSHLMGMFYRTIRMME NGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQ AQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI PYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFG **A**PVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFV DLCILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLD PNKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEP NEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRL DDFFKVTGSLSSAKRKEPEPKGSTKKKAKTGAAGKFK RGK	SEQ ID NO: 200
FEN1 K354R (relative to FEN1 wt)	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSI YQFLIAVRQGGDVLQNEEGETTSHLMGMFYRTIRMME NGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQ AQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI PYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGS PVLMRHLTASEAKKLPIQEFHLSRILQELGLNQEQFVD LCILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLDP NKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSEPN EEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLD DFFKVTGSLSSA**R**RKEPEPKGSTKKKAKTGAAGKFKR GK	SEQ ID NO: 201
GEN1	MGVNDLWQILEPVKQHIPLRNLGGKTIAVDLSLWVCE AQTVKKMMGSVMKPHLRNLFFRISYLTQMDVKLVFV MEGEPPKLKADVISKRNQSRYGSSGKSWSQKTGRSHF KSVLRECLHMLECLGIPWVQAAGEAEAMCAYLNAGG HVDGCLTNDGDTFLYGAQTVYRNFTMNTKDPHVDCY TMSSIKSKLGLDRDALVGLAILLGCDYLPKGVPGVGKE QALKLIQILKGQSLLQRFNRWNETSCNSSPQLLVTKKL AHCSVCSHPGSPKDHERNGCRLCKSDKYCEPHDYEYC CPCEWHRTEHDRQLSEVENNIKKKACCCEGFPFHEVIQ EFLLNKDKLVKVIRYQRPDLLLFQRFTLEKMEWPNHY ACEKLLVLLTHYDMIERKLGSRNSNQLQPIRIVKTRIRN GVHCFEIEWEKPEHYAMEDKQHGEFALLTIEEESLFEA AYPEIVAVYQKQKLEIKGKKQKRIKPKENNLPEPDEVM SFQSHMTLKPTCEIFHKQNSKLNSGISPDPTLPQESISAS LNSLLLPKNTPCLNAQEQFMSSLRPLAIQQIKAVSKSLI SESSQPNTSSHNISVIADLHLSTIDWEGTSFSNSPAIQRN	SEQ ID NO: 202

	TFSHDLKSEVESELSAIPDGFENIPEQLSCESERYTANIK KVLDEDSDGISPEEHLLSGITDLCLQDLPLKERIFTKLSY PQDNLQPDVNLKTLSILSVKESCIANSGSDCTSHLSKDL PGIPLQNESRDSKILKGDQLLQEDYKVNTSVPYSVSNT VVKTCNVRPPNTALDHSRKVDMQTTRKILMKKSVCLD RHSSDEQSAPVFGKAKYTTQRMKHSSQKHNSSHFKES GHNKLSSPKIHIKETEQCVRSYETAENEESCFPDSTKSS LSSLQCHKKENNSGTCLDSPLPLRQRLKLRFQST	
ERCC5	MGVQGLWKLLECSGRQVSPEALEGKILAVDISIWLNQ ALKGVRDRHGNSIENPHLLTLFHRLCKLLFFRIRPIFVF DGDAPLLKKQTLVKRRQRKDLASSDSRKTTEKLLKTF LKRQAIKTAFRSKRDEALPSLTQVRRENDLYVLPPLQE EEKHSSEEEDEKEWQERMNQKQALQEEFFHNPQAIDIE SEDFSSLPPEVKHEILTDMKEFTKRRRTLFEAMPEESDD FSQYQLKGLLKKNYLNQHIEHVQKEMNQQHSGHIRRQ YEDEGGFLKEVESRRVVSEDTSHYILIKGIQAKTVAEV DSESLPSSSKMHGMSFDVKSSPCEKLKTEKEPDATPPSP RTLLAMQAALLGSSSEEELESENRRQARGRNAPAAVD EGSISPRTLSAIKRALDDDEDVKVCAGDDVQTGGPGAE EMRINSSTENSDEGLKVRDGKGIPFTATLASSSVNSAEE HVASTNEGREPTDSVPKEQMSLVHVGTEAFPISDESMI KDRKDRLPLESAVVRHSDAPGLPNGRELTPASPTCTNS VSKNETHAEVLEQQNELCPYESKFDSSLLSSDDETKCK PNSASEVIGPVSLQETSSIVSVPSEAVDNVENVVSFNAK EHENFLETIQEQQTTESAGQDLISIPKAVEPMEIDSEESE SDGSFIEVQSVISDEELQAEFPETSKPPSEQGEEELVGTR EGEAPAESESLLRDNSERDDVDGEPQEAEKDAEDSLHE WQDINLEELETLESNLLAQQNSLKAQKQQQERIAATVT GQMFLESQELLRLFGIPYIQAPMEAEAQCAILDLTDQTS GTITDDSDIWLFGARHVYRNFFNKNKFVEYYQYVDFH NQLGLDRNKLINLAYLLGSDYTEGIPTVGCVTAMEILN EFPGHGLEPLLKFSEWWHEAQKNPKIRPNPHDTKVKK KLRTLQLTPGFPNPAVAEAYLKPVVDDSKGSFLWGKP DLDKIREFCQRYFGWNRTKTDESLFPVLKQLDAQQTQ LRIDSFFRLAQQEKEDAKRIKSQRLNRAVTCMLRKEKE AAASEIEAVSVAMEKEFELLDKAKRKTQKRGITNTLEE SSSLKRKRLSDSKRKNTCGGFLGETCLSESSDGSSSEDA ESSSLMNVQRRTAAKEPKTSASDSQNSVKEAPVKNGG ATTSSSSDSDDDGGKEKMVLVTARSVFGKKRRKLRRA RGRKRKT	SEQ ID NO: 203

[0557] In various embodiments, the prime editor fusion proteins contemplated herein may include any flap endonulcease variant of the above-disclosed sequences having an amino acid sequence that is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97%

identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to any of the above sequences.

[0558] Other endonucleases that may be utilized by the instant methods to facilitate removal of the 5' end single strand DNA flap include, but are not limited to (1) trex 2, (2) exo1 endonuclease (e.g., Keijzers et al., *Biosci Rep.* 2015, 35(3): e00206)

Trex 2

[0559] 3' three prime repair exonuclease 2 (TREX2) - human

[0560] Accession No. NM_080701

MSEAPRAETFVFLDLEATGLPSVEPEIAELSLFAVHRSSLENPEHDESGALVLPRVLD KLTLCMCPERPFTAKASEITGLSSEGLARCRKAGFDGAVVRTLQAFLSRQAGPICLVA HNGFDYDFPLLCAELRRLGARLPRDTVCLDTLPALRGLDRAHSHGTRARGRQGYSL GSLFHRYFRAEPSAAHSAEGDVHTLLLIFLHRAAELLAWADEQARGWAHIEPMYLPP DDPSLEA (SEQ ID NO: 3865).

[0561] 3' three prime repair exonuclease 2 (TREX2) - mouse

[0562] Accession No. NM_011907

[0563] MSEPPRAETFVFLDLEATGLPNMDPEIAEISLFAVHRSSLENPERDDSGSLVLP RVLDKLTLCMCPERPFTAKASEITGLSSESLMHCGKAGFNGAVVRTLQGFLSRQEGPI CLVAHNGFDYDFPLLCTELQRLGAHLPQDTVCLDTLPALRGLDRAHSHGTRAQGRK SYSLASLFHRYFQAEPSAAHSAEGDVHTLLLIFLHRAPELLAWADEQARSWAHIEPM YVPPDGPSLEA (SEQ ID NO: 3866).

[0564] 3' three prime repair exonuclease 2 (TREX2) - rat

[0565] Accession No. NM_001107580

[0566] MSEPLRAETFVFLDLEATGLPNMDPEIAEISLFAVHRSSLENPERDDSGSLVLP RVLDKLTLCMCPERPFTAKASEITGLSSEGLMNCRKAAFNDAVVRTLQGFLSRQEGP ICLVAHNGFDYDFPLLCTELQRLGAHLPRDTVCLDTLPALRGLDRVHSHGTRAQGRK SYSLASLFHRYFQAEPSAAHSAEGDVNTLLLIFLHRAPELLAWADEQARSWAHIEPM YVPPDGPSLEA (SEQ ID NO: 3867).

ExoI

[0567] Human exonuclease 1 (EXO1) has been implicated in many different DNA metabolic processes, including DNA mismatch repair (MMR), micro-mediated end-joining, homologous recombination (HR), and replication. Human EXO1 belongs to a family of eukaryotic nucleases, Rad2/XPG, which also include FEN1 and GEN1. The Rad2/XPG family is conserved in the nuclease domain through species from phage to human. The EXO1

gene product exhibits both 5′ exonuclease and 5′ flap activity. Additionally, EXO1 contains an intrinsic 5′ RNase H activity. Human EXO1 has a high affinity for processing double stranded DNA (dsDNA), nicks, gaps, pseudo Y structures and can resolve Holliday junctions using its inherit flap activity. Human EXO1 is implicated in MMR and contain conserved binding domains interacting directly with MLH1 and MSH2. EXO1 nucleolytic activity is positively stimulated by PCNA, MutSα (MSH2/MSH6 complex), 14-3-3, MRN and 9-1-1 complex.

[0568] exonuclease 1 (EXO1) Accession No. NM_003686 (Homo sapiens exonuclease 1 (EXO1), transcript variant 3) – isoform A

MGIQGLLQFIKEASEPIHVRKYKGQVVAVDTYCWLHKGAIACAEKLAKGEPTDRYV GFCMKFVNMLLSHGIKPILVFDGCTLPSKKEVERSRRERRQANLLKGKQLLREGKVS EARECFTRSINITHAMAHKVIKAARSQGVDCLVAPYEADAQLAYLNKAGIVQAIITE DSDLLAFGCKKVILKMDQFGNGLEIDQARLGMCRQLGDVFTEEKFRYMCILSGCDY LSSLRGIGLAKACKVLRLANNPDIVKVIKKIGHYLKMNITVPEDYINGFIRANNTFLY QLVFDPIKRKLIPLNAYEDDVDPETLSYAGQYVDDSIALQIALGNKDINTFEQIDDYN PDTAMPAHSRSHSWDDKTCQKSANVSSIWHRNYSPRPESGTVSDAPQLKENPSTVG VERVISTKGLNLPRKSSIVKRPRSAELSEDDLLSQYSLSFTKKTKKNSSEGNKSLSFSE VFVPDLVNGPTNKKSVSTPPRTRNKFATFLQRKNEESGAVVVPGTRSRFFCSSDSTDC VSNKVSIQPLDETAVTDKENNLHESEYGDQEGKRLVDTDVARNSSDDIPNNHIPGDH IPDKATVFTDEESYSFESSKFTRTISPPTLGTLRSCFSWSGGLGDFSRTPSPSPSTALQQ FRRKSDSPTSLPENNMSDVSQLKSEESSDDESHPLREEACSSQSQESGEFSLQSSNASK LSQCSSKDSDSEESDCNIKLLDSQSDQTSKLRLSHFSKKDTPLRNKVPGLYKSSSADS LSTTKIKPLGPARASGLSKKPASIQKRKHHNAENKPGLQIKLNELWKNFGFKKF (SEQ ID NO: 3868).

[0569] exonuclease 1 (EXO1) Accession No. NM_006027 (Homo sapiens exonuclease 1 (EXO1), transcript variant 3) – isoform B

[0570] MGIQGLLQFIKEASEPIHVRKYKGQVVAVDTYCWLHKGAIACAEKLAKGEPT DRYVGFCMKFVNMLLSHGIKPILVFDGCTLPSKKEVERSRRERRQANLLKGKQLLRE GKVSEARECFTRSINITHAMAHKVIKAARSQGVDCLVAPYEADAQLAYLNKAGIVQ AIITEDSDLLAFGCKKVILKMDQFGNGLEIDQARLGMCRQLGDVFTEEKFRYMCILS GCDYLSSLRGIGLAKACKVLRLANNPDIVKVIKKIGHYLKMNITVPEDYINGFIRANN TFLYQLVFDPIKRKLIPLNAYEDDVDPETLSYAGQYVDDSIALQIALGNKDINTFEQID DYNPDTAMPAHSRSHSWDDKTCQKSANVSSIWHRNYSPRPESGTVSDAPQLKENPS

TVGVERVISTKGLNLPRKSSIVKRPRSAELSEDDLLSQYSLSFTKKTKKNSSEGNKSLS
FSEVFVPDLVNGPTNKKSVSTPPRTRNKFATFLQRKNEESGAVVVPGTRSRFFCSSDS
TDCVSNKVSIQPLDETAVTDKENNLHESEYGDQEGKRLVDTDVARNSSDDIPNNHIP
GDHIPDKATVFTDEESYSFESSKFTRTISPPTLGTLRSCFSWSGGLGDFSRTPSPSPSTA
LQQFRRKSDSPTSLPENNMSDVSQLKSEESSDDESHPLREEACSSQSQESGEFSLQSSN
ASKLSQCSSKDSDSEESDCNIKLLDSQSDQTSKLRLSHFSKKDTPLRNKVPGLYKSSS
ADSLSTTKIKPLGPARASGLSKKPASIQKRKHHNAENKPGLQIKLNELWKNFGFKKD
SEKLPPCKKPLSPVRDNIQLTPEAEEDIFNKPECGRVQRAIFQ (SEQ ID NO: 3869).

[0571] exonuclease 1 (EXO1) Accession No. NM_001319224 (Homo sapiens exonuclease 1 (EXO1), transcript variant 4) – isoform C

[0572] MGIQGLLQFIKEASEPIHVRKYKGQVVAVDTYCWLHKGAIACAEKLAKGEPT
DRYVGFCMKFVNMLLSHGIKPILVFDGCTLPSKKEVERSRRERRQANLLKGKQLLRE
GKVSEARECFTRSINITHAMAHKVIKAARSQGVDCLVAPYEADAQLAYLNKAGIVQ
AIITEDSDLLAFGCKKVILKMDQFGNGLEIDQARLGMCRQLGDVFTEEKFRYMCILS
GCDYLSSLRGIGLAKACKVLRLANNPDIVKVIKKIGHYLKMNITVPEDYINGFIRANN
TFLYQLVFDPIKRKLIPLNAYEDDVDPETLSYAGQYVDDSIALQIALGNKDINTFEQID
DYNPDTAMPAHSRSHSWDDKTCQKSANVSSIWHRNYSPRPESGTVSDAPQLKENPS
TVGVERVISTKGLNLPRKSSIVKRPRSELSEDDLLSQYSLSFTKKTKKNSSEGNKSLSF
SEVFVPDLVNGPTNKKSVSTPPRTRNKFATFLQRKNEESGAVVVPGTRSRFFCSSDST
DCVSNKVSIQPLDETAVTDKENNLHESEYGDQEGKRLVDTDVARNSSDDIPNNHIPG
DHIPDKATVFTDEESYSFESSKFTRTISPPTLGTLRSCFSWSGGLGDFSRTPSPSPSTAL
QQFRRKSDSPTSLPENNMSDVSQLKSEESSDDESHPLREEACSSQSQESGEFSLQSSN
ASKLSQCSSKDSDSEESDCNIKLLDSQSDQTSKLRLSHFSKKDTPLRNKVPGLYKSSS
ADSLSTTKIKPLGPARASGLSKKPASIQKRKHHNAENKPGLQIKLNELWKNFGFKKD
SEKLPPCKKPLSPVRDNIQLTPEAEEDIFNKPECGRVQRAIFQ (SEQ ID NO: 3870).

D. Inteins and split-inteins

[0573] It will be understood that in some embodiments (e.g., delivery of a prime editor *in vivo* using AAV particles), it may be advantageous to split a polypeptide (e.g., a deaminase or a napDNAbp) or a fusion protein (e.g., a prime editor) into an N-terminal half and a C-terminal half, delivery them separately, and then allow their colocalization to reform the complete protein (or fusion protein as the case may be) within the cell. Separate halves of a protein or a fusion protein may each comprise a split-intein tag to facilitate the reformation of the complete protein or fusion protein by the mechanism of protein trans splicing.

[0574] Protein trans-splicing, catalyzed by split inteins, provides an entirely enzymatic method for protein ligation. A split-intein is essentially a contiguous intein (e.g. a mini-intein) split into two pieces named N-intein and C-intein, respectively. The N-intein and C-intein of a split intein can associate non-covalently to form an active intein and catalyze the splicing reaction essentially in same way as a contiguous intein does. Split inteins have been found in nature and also engineered in laboratories. As used herein, the term "split intein" refers to any intein in which one or more peptide bond breaks exists between the N-terminal and C-terminal amino acid sequences such that the N-terminal and C-terminal sequences become separate molecules that can non-covalently reassociate, or reconstitute, into an intein that is functional for trans-splicing reactions. Any catalytically active intein, or fragment thereof, may be used to derive a split intein for use in the methods of the invention. For example, in one aspect the split intein may be derived from a eukaryotic intein. In another aspect, the split intein may be derived from a bacterial intein. In another aspect, the split intein may be derived from an archaeal intein. Preferably, the split intein so-derived will possess only the amino acid sequences essential for catalyzing trans-splicing reactions.

[0575] As used herein, the "N-terminal split intein (In)" refers to any intein sequence that comprises an N- terminal amino acid sequence that is functional for trans-splicing reactions. An In thus also comprises a sequence that is spliced out when trans-splicing occurs. An In can comprise a sequence that is a modification of the N-terminal portion of a naturally occurring intein sequence. For example, an In can comprise additional amino acid residues and/or mutated residues so long as the inclusion of such additional and/or mutated residues does not render the In non-functional in trans-splicing. Preferably, the inclusion of the additional and/or mutated residues improves or enhances the trans-splicing activity of the In.

[0576] As used herein, the "C-terminal split intein (Ic)" refers to any intein sequence that comprises a C- terminal amino acid sequence that is functional for trans-splicing reactions. In one aspect, the Ic comprises 4 to 7 contiguous amino acid residues, at least 4 amino acids of which are from the last β-strand of the intein from which it was derived. An Ic thus also comprises a sequence that is spliced out when trans-splicing occurs. An Ic can comprise a sequence that is a modification of the C-terminal portion of a naturally occurring intein sequence. For example, an Ic can comprise additional amino acid residues and/or mutated residues so long as the inclusion of such additional and/or mutated residues does not render the In non-functional in trans-splicing. Preferably, the inclusion of the additional and/or mutated residues improves or enhances the trans-splicing activity of the Ic.

[0577] In some embodiments of the invention, a peptide linked to an Ic or an In can comprise an additional chemical moiety including, among others, fluorescence groups, biotin, polyethylene glycol (PEG), amino acid analogs, unnatural amino acids, phosphate groups, glycosyl groups, radioisotope labels, and pharmaceutical molecules. In other embodiments, a peptide linked to an Ic can comprise one or more chemically reactive groups including, among others, ketone, aldehyde, Cys residues and Lys residues. The N-intein and C-intein of a split intein can associate non-covalently to form an active intein and catalyze the splicing reaction when an "intein-splicing polypeptide (ISP)" is present. As used herein, "intein-splicing polypeptide (ISP)" refers to the portion of the amino acid sequence of a split intein that remains when the Ic, In, or both, are removed from the split intein. In certain embodiments, the In comprises the ISP. In another embodiment, the Ic comprises the ISP. In yet another embodiment, the ISP is a separate peptide that is not covalently linked to In nor to Ic.

[0578] Split inteins may be created from contiguous inteins by engineering one or more split sites in the unstructured loop or intervening amino acid sequence between the -12 conserved beta-strands found in the structure of mini-inteins. Some flexibility in the position of the split site within regions between the beta-strands may exist, provided that creation of the split will not disrupt the structure of the intein, the structured beta-strands in particular, to a sufficient degree that protein splicing activity is lost.

[0579] In protein trans-splicing, one precursor protein consists of an N-extein part followed by the N-intein, another precursor protein consists of the C-intein followed by a C-extein part, and a trans-splicing reaction (catalyzed by the N- and C-inteins together) excises the two intein sequences and links the two extein sequences with a peptide bond. Protein trans-splicing, being an enzymatic reaction, can work with very low (e.g. micromolar) concentrations of proteins and can be carried out under physiological conditions.

[0580] Exemplary sequences are as follows:

NAME	SEQUENCE OF LIGAND-DEPENDENT INTEIN
2-4 INTEIN:	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD

	KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 8)
3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDR VAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEIL MIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATS SRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRAL DKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHL YSMKYTNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDK FLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHN C (SEQ ID NO: 9)
30R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 10)
30R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 11)
30R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 12)
37R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYNPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL

	ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC ((SEQ ID NO: 13)
37R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 14)
37R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 15)

[0581] Although inteins are most frequently found as a contiguous domain, some exist in a naturally split form. In this case, the two fragments are expressed as separate polypeptides and must associate before splicing takes place, so-called protein trans-splicing.

[0582] An exemplary split intein is the *Ssp* DnaE intein, which comprises two subunits, namely, DnaE-N and DnaE-C. The two different subunits are encoded by separate genes, namely *dnaE-n* and *dnaE-c*, which encode the DnaE-N and DnaE-C subunits, respectively. DnaE is a naturally occurring split intein in *Synechocytis sp.* PCC6803 and is capable of directing trans-splicing of two separate proteins, each comprising a fusion with either DnaE-N or DnaE-C.

[0583] Additional naturally occurring or engineered split-intein sequences are known in the or can be made from whole-intein sequences described herein or those available in the art. Examples of split-intein sequences can be found in Stevens et al., "A promiscuous split intein with expanded protein engineering applications," PNAS, 2017, Vol.114: 8538-8543; Iwai et al., "Highly efficient protein trans-splicing by a naturally split DnaE intein from Nostc punctiforme, FEBS Lett, 580: 1853-1858, each of which are incorporated herein by reference. Additional split intein sequences can be found, for example, in WO 2013/045632, WO

2014/055782, WO 2016/069774, and EP2877490, the contents each of which are incorporated herein by reference.

[0584] In addition, protein splicing in trans has been described *in vivo* and *in vitro* (Shingledecker, et al., *Gene* 207:187 (1998), Southworth, et al., *EMBO J.* 17:918 (1998); Mills, et al., *Proc. Natl. Acad. Sci. USA,* 95:3543-3548 (1998); Lew, et al., *J. Biol. Chem.,* 273:15887-15890 (1998); Wu, et al., *Biochim. Biophys. Acta* 35732:1 (1998b), Yamazaki, et al., *J. Am. Chem. Soc.* 120:5591 (1998), Evans, et al., *J. Biol. Chem.* 275:9091 (2000); Otomo, et al., *Biochemistry* 38:16040-16044 (1999); Otomo, et al., *J. Biolmol. NMR* 14:105-114 (1999); Scott, et al., *Proc. Natl. Acad. Sci. USA* 96:13638-13643 (1999)) and provides the opportunity to express a protein as to two inactive fragments that subsequently undergo ligation to form a functional product, e.g., as shown in FIGs. 66 and 67 with regard to the formation of a complete PE fusion protein from two separately-expressed halves.

E. RNA-protein interaction domain

[0585] In various embodiments, two separate protein domains (e.g., a Cas9 domain and a polymerase domain) may be colocalized to one another to form a functional complex (akin to the function of a fusion protein comprising the two separate protein domains) by using an "RNA-protein recruitment system," such as the "MS2 tagging technique." Such systems generally tag one protein domain with an "RNA-protein interaction domain" (aka "RNA-protein recruitment domain") and the other with an "RNA-binding protein" that specifically recognizes and binds to the RNA-protein interaction domain, e.g., a specific hairpin structure. These types of systems can be leveraged to colocalize the domains of a prime editor, as well as to recruitment additional functionalities to a prime editor, such as a UGI domain. In one example, the MS2 tagging technique is based on the natural interaction of the MS2 bacteriophage coat protein ("MCP" or "MS2cp") with a stem-loop or hairpin structure present in the genome of the phage, i.e., the "MS2 hairpin." In the case of the MS2 hairpin, it is recognized and bound by the MS2 bacteriophage coat protein (MCP). Thus, in one exemplarly scenario a deaminase-MS2 fusion can recruit a Cas9-MCP fusion.

[0586] A review of other modular RNA-protein interaction domains are described in the art, for example, in Johansson et al., "RNA recognition by the MS2 phage coat protein," *Sem Virol.*, 1997, Vol. 8(3): 176-185; Delebecque et al., "Organization of intracellular reactions with rationally designed RNA assemblies," *Science*, 2011, Vol. 333: 470-474; Mali et al., "Cas9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.*, 2013, Vol.31: 833-838; and Zalatan et

al., "Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds," *Cell*, 2015, Vol.160: 339-350, each of which are incorporated herein by reference in their entireties. Other systems include the PP7 hairpin, which specifically recruits the PCP protein, and the "com" hairpin, which specifically recruits the Com protein. See Zalatan et al.

[0587] The nucleotide sequence of the MS2 hairpin (or equivalently referred to as the "MS2 aptamer") is: GCCAACATGAGGATCACCCATGTCTGCAGGGCC (SEQ ID NO: 3871).

[0588] The amino acid sequence of the MCP or MS2cp is: GSASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCSVRQSSAQ NRKYTIKVEVPKVATQTVGGEELPVAGWRSYLNMELTIPIFATNSDCELIVKAMQGL LKDGNPIPSAIAANSGIY (SEQ ID NO: 3872).

F. UGI domain

[0589] In other embodiments, the prime editors described herein may comprise one or more uracil glycosylase inhibitor domains. The term "uracil glycosylase inhibitor (UGI)" or "UGI domain," as used herein, refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a UGI as set forth in SEQ ID NO: 3873. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. For example, in some embodiments, a UGI domain comprises a fragment of the amino acid sequence set forth in SEQ ID NO: 3873. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid sequence as set forth in SEQ ID NO: 3873. In some embodiments, a UGI comprises an amino acid sequence homologous to the amino acid sequence set forth in SEQ ID NO: 3873, or an amino acid sequence homologous to a fragment of the amino acid sequence set forth in SEQ ID NO: 3873. In some embodiments, proteins comprising UGI or fragments of UGI or homologs of UGI or UGI fragments are referred to as "UGI variants." A UGI variant shares homology to UGI, or a fragment thereof. For example a UGI variant is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to a wild type UGI or a UGI as set forth in SEQ ID NO: 3873. In some embodiments, the UGI variant comprises a fragment of UGI, such that the fragment is at least 70% identical, at least 80% identical, at least 90%

identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% to the corresponding fragment of wild-type UGI or a UGI as set forth in SEQ ID NO: 3873. In some embodiments, the UGI comprises the following amino acid sequence:

[0590] Uracil-DNA glycosylase inhibitor:

[0591] >splP14739lUNGI_BPPB2
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDESTDENVMLLT
SDAPEYKPWALVIQDSNGENKIKML (SEQ ID NO: 3873).

[0592] The prime editors described herein may comprise more than one UGI domain, which may be separated by one or more linkers as described herein.

G. Additional PE elements

[0593] In certain embodiments, the prime editors described herein may comprise an inhibitor of base repair. The term "inhibitor of base repair" or "IBR" refers to a protein that is capable in inhibiting the activity of a nucleic acid repair enzyme, for example a base excision repair enzyme. In some embodiments, the IBR is an inhibitor of OGG base excision repair. In some embodiments, the IBR is an inhibitor of base excision repair ("iBER"). Exemplary inhibitors of base excision repair include inhibitors of APE1, Endo III, Endo IV, Endo V, Endo VIII, Fpg, hOGG1, hNEIL1, T7 EndoI, T4PDG, UDG, hSMUG1, and hAAG. In some embodiments, the IBR is an inhibitor of Endo V or hAAG. In some embodiments, the IBR is an iBER that may be a catalytically inactive glycosylase or catalytically inactive dioxygenase or a small molecule or peptide inhibitor of an oxidase, or variants threreof. In some embodiments, the IBR is an iBER that may be a TDG inhibitor, MBD4 inhibitor or an inhibitor of an AlkBH enzyme. In some embodiments, the IBR is an iBER that comprises a catalytically inactive TDG or catalytically inactive MBD4. An exemplary catalytically inactive TDG is an N140A mutant of SEQ ID NO: 3872 (human TDG).

[0594] Some exemplary glycosylases are provided below. The catalytically inactivated variants of any of these glycosylase domains are iBERs that may be fused to the napDNAbp or polymerase domain of the prime editors provided in this disclosure.

[0595] OGG (human)

[0596] MPARALLPRRMGHRTLASTPALWASIPCPRSELRLDLVLPSGQSFRWREQSPA
HWSGVLADQVWTLTQTEEQLHCTVYRGDKSQASRPTPDELEAVRKYFQLDVTLAQ
LYHHWGSVDSHFQEVAQKFQGVRLLRQDPIECLFSFICSSNNNIARITGMVERLCQAF
GPRLIQLDDVTYHGFPSLQALAGPEVEAHLRKLGLGYRARYVSASARAILEEQGGLA

WLQQLRESSYEEAHKALCILPGVGTKVADCICLMALDKPQAVPVDVHMWHIAQRD
YSWHPTTSQAKGPSPQTNKELGNFFRSLWGPYAGWAQAVLFSADLRQSRHAQEPPA
KRRKGSKGPEG (SEQ ID NO: 3869)

[0597] MPG (human)

[0598] MVTPALQMKKPKQFCRRMGQKKQRPARAGQPHSSSDAAQAPAEQPHSSSDA
AQAPCPRERCLGPPTTPGPYRSIYFSSPKGHLTRLGLEFFDQPAVPLARAFLGQVLVR
RLPNGTELRGRIVETEAYLGPEDEAAHSRGGRQTPRNRGMFMKPGTLYVYIIYGMYF
CMNISSQGDGACVLLRALEPLEGLETMRQLRSTLRKGTASRVLKDRELCSGPSKLCQ
ALAINKSFDQRDLAQDEAVWLERGPLEPSEPAVVAAARVGVGHAGEWARKPLRFY
VRGSPWVSVVDRVAEQDTQA (SEQ ID NO: 3870)

[0599] MBD4 (human)

[0600] MGTTGLESLSLGDRGAAPTVTSSERLVPDPPNDLRKEDVAMELERVGEDEEQ
MMIKRSSECNPLLQEPIASAQFGATAGTECRKSVPCGWERVVKQRLFGKTAGRFDV
YFISPQGLKFRSKSSLANYLHKNGETSLKPEDFDFTVLSKRGIKSRYKDCSMAALTSH
LQNQSNNSNWNLRTRSKCKKDVFMPPSSSSELQESRGLSNFTSTHLLLKEDEGVDDV
NFRKVRKPKGKVTILKGIPIKKTKKGCRKSCSGFVQSDSKRESVCNKADAESEPVAQ
KSQLDRTVCISDAGACGETLSVTSEENSLVKKKERSLSSGSNFCSEQKTSGIINKFCSA
KDSEHNEKYEDTFLESEEIGTKVEVVERKEHLHTDILKRGSEMDNNCSPTRKDFTGE
KIFQEDTIPRTQIERRKTSLYFSSKYNKEALSPPRRKAFKKWTPPRSPFNLVQETLFHD
PWKLLIATIFLNRTSGKMAIPVLWKFLEKYPSAEVARTADWRDVSELLKPLGLYDLR
AKTIVKFSDEYLTKQWKYPIELHGIGKYGNDSYRIFCVNEWKQVHPEDHKLNKYHD
WLWENHEKLSLS (SEQ ID NO: 3871)

[0601] TDG (human)

[0602] MEAENAGSYSLQQAQAFYTFPFQQLMAEAPNMAVVNEQQMPEEVPAPAPA
QEPVQEAPKGRKRKPRTTEPKQPVEPKKPVESKKSGKSAKSKEKQEKITDTFKVKRK
VDRFNGVSEAELLTKTLPDILTFNLDIVIIGINPGLMAAYKGHHYPGPGNHFWKCLFM
SGLSEVQLNHMDDHTLPGKYGIGFTNMVERTTPGSKDLSSKEFREGGRILVQKLQKY
QPRIAVFNGKCIYEIFSKEVFGVKVKNLEFGLQPHKIPDTETLCYVMPSSSARCAQFPR
AQDKVHYYIKLKDLRDQLKGIERNMDVQEVQYTFDLQLAQEDAKKMAVKEEKYDP
GYEAAYGGAYGENPCSSEPCGFSSNGLIESVELRGESAFSGIPNGQWMTQSFTDQIPS
FSNHCGTQEQEEESHA (SEQ ID NO: 3872)

[0603] In some embodiments, the fusion proteins described herein may comprise one or more heterologous protein domains (e.g., about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or

more domains in addition to the prime editor components). A fusion protein may comprise any additional protein sequence, and optionally a linker sequence between any two domains. Other exemplary features that may be present are localization sequences, such as cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the fusion proteins.

[0604] Examples of protein domains that may be fused to a prime editor or component thereof (e.g., the napDNAbp domain, the polymerase domain, or the NLS domain) include, without limitation, epitope tags, and reporter gene sequences. Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-5-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT), beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). A prime editor may be fused to a gene sequence encoding a protein or a fragment of a protein that bind DNA molecules or bind other cellular molecules, including, but not limited to, maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. Additional domains that may form part of a prime editor are described in US Patent Publication No. 2011/0059502, published March 10, 2011 and incorporated herein by reference in its entirety.

[0605] In an aspect of the disclosure, a reporter gene which includes, but is not limited to, glutathione-5-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP), may be introduced into a cell to encode a gene product which serves as a marker by which to measure the alteration or modification of expression of the gene product. In certain embodiments of the disclosure the gene product is luciferase. In a further embodiment of the disclosure the expression of the gene product is decreased.

[0606] Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags,

hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (e.g., Softag 1, Softag 3), strep-tags , biotin ligase tags, FlAsH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the fusion protein comprises one or more His tags.

[0607] In some embodiments of the present disclosure, the activity of the prime editing system may be temporally regulated by adjusting the residence time, the amount, and/or the activity of the expressed components of the PE system. For example, as described herein, the PE may be fused with a protein domain that is capable of modifying the intracellular half-life of the PE. In certain embodiments involving two or more vectors (e.g., a vector system in which the components described herein are encoded on two or more separate vectors), the activity of the PE system may be temporally regulated by controlling the timing in which the vectors are delivered. For example, in some embodiments a vector encoding the nuclease system may deliver the PE prior to the vector encoding the template. In other embodiments, the vector encoding the PEgRNA may deliver the guide prior to the vector encoding the PE system. In some embodiments, the vectors encoding the PE system and PEgRNA are delivered simultaneously. In certain embodiments, the simultaneously delivered vectors temporally deliver, e.g., the PE, PEgRNA, and/or second strand guide RNA components. In further embodiments, the RNA (such as, e.g., the nuclease transcript) transcribed from the coding sequence on the vectors may further comprise at least one element that is capable of modifying the intracellular half-life of the RNA and/or modulating translational control. In some embodiments, the half-life of the RNA may be increased. In some embodiments, the half-life of the RNA may be decreased. In some embodiments, the element may be capable of increasing the stability of the RNA. In some embodiments, the element may be capable of decreasing the stability of the RNA. In some embodiments, the element may be within the 3' UTR of the RNA. In some embodiments, the element may include a polyadenylation signal (PA). In some embodiments, the element may include a cap, e.g., an upstream mRNA or PEgRNA end. In some embodiments, the RNA may comprise no PA such that it is subject to quicker degradation in the cell after transcription. In some embodiments, the element may include at least one AU-rich element (ARE). The AREs may be bound by ARE binding proteins (ARE-BPs) in a manner that is dependent upon tissue type, cell type, timing, cellular localization, and environment. In some embodiments the destabilizing element may promote

RNA decay, affect RNA stability, or activate translation. In some embodiments, the ARE may comprise 50 to 150 nucleotides in length. In some embodiments, the ARE may comprise at least one copy of the sequence AUUUA. In some embodiments, at least one ARE may be added to the 3' UTR of the RNA. In some embodiments, the element may be a Woodchuck Hepatitis Virus (WHP).

[0608] Posttranscriptional Regulatory Element (WPRE), which creates a tertiary structure to enhance expression from the transcript. In further embodiments, the element is a modified and/or truncated WPRE sequence that is capable of enhancing expression from the transcript, as described, for example in Zufferey et al., J Virol, 73(4): 2886-92 (1999) and Flajolet et al., J Virol, 72(7): 6175-80 (1998). In some embodiments, the WPRE or equivalent may be added to the 3' UTR of the RNA. In some embodiments, the element may be selected from other RNA sequence motifs that are enriched in either fast- or slow-decaying transcripts.

[0609] In some embodiments, the vector encoding the PE or the PEgRNA may be self-destroyed via cleavage of a target sequence present on the vector by the PE system. The cleavage may prevent continued transcription of a PE or a PEgRNA from the vector. Although transcription may occur on the linearized vector for some amount of time, the expressed transcripts or proteins subject to intracellular degradation will have less time to produce off-target effects without continued supply from expression of the encoding vectors.

[6] PEgRNAs

[0610] The prime editing system described herein contemplates the use of any suitable PEgRNAs. The inventors have discovered that the mechanism of target-primed reverse transcription (TPRT) can be leveraged or adapted for conducting precision and versatile CRISPR/Cas-based genome editing through the use of a specially configured guide RNA comprising a reverse transcription (RT) template sequence that codes for the desired nucleotide change. The application refers to this specially configured guide RNA as an "extended guide RNA" or a "PEgRNA" since the RT template sequence can be provided as an extension of a standard or traditional guide RNA molecule. The application contemplates any suitable configuration or arrangement for the extended guide RNA.

PEgRNA architecture

[0611] FIG. 3A shows one embodiment of an extended guide RNA usable in the prime editing system disclosed herein whereby a traditional guide RNA (the green portion) includes a ~20 nt protospacer sequence and a gRNA core region, which binds with the napDNAbp. In this embodiment, the guide RNA includes an extended RNA segment at the 5′ end, i.e., a 5′

extension. In this embodiment, the 5′extension includes a reverse transcription template sequence, a reverse transcription primer binding site, and an optional 5-20 nucleotide linker sequence. As shown in FIGs. 1A-1B, the RT primer binding site hybrizes to the free 3′ end that is formed after a nick is formed in the non-target strand of the R-loop, thereby priming reverse transcriptase for DNA polymerization in the 5′-3′ direction.

[0612] FIG. 3B shows another embodiment of an extended guide RNA usable in the prime editing system disclosed herein whereby a traditional guide RNA (the green portion) includes a ~20 nt protospacer sequence and a gRNA core, which binds with the napDNAbp. In this embodiment, the guide RNA includes an extended RNA segment at the 3′ end, i.e., a 3′ extension. In this embodiment, the 3′extension includes a reverse transcription template sequence, and a reverse transcription primer binding site. As shown in FIGs. 1C-1D, the RT primer binding site hybrizes to the free 3′ end that is formed after a nick is formed in the non-target strand of the R-loop, thereby priming reverse transcriptase for DNA polymerization in the 5′-3′ direction.

[0613] FIG. 3C shows another embodiment of an extend guide RNA usable in the prime editing system disclosed herein whereby a traditional guide RNA (the green portion) includes a ~20 nt protospacer sequence and a gRNA core, which binds with the napDNAbp. In this embodiment, the guide RNA includes an extended RNA segment at an intermolecular position within the gRNA core, i.e., an intramolecular extension. In this embodiment, the intramolecular extension includes a reverse transcription template sequence, and a reverse transcription primer binding site. The RT primer binding site hybrizes to the free 3′ end that is formed after a nick is formed in the non-target strand of the R-loop, thereby priming reverse transcriptase for DNA polymerization in the 5′-3′ direction.

[0614] In one embodiment, the position of the intermolecular RNA extension is not in the protospacer sequence of the guide RNA. In another embodiment, the position of the intermolecular RNA extension in the gRNA core. In still another embodiment, the position of the intermolecular RNA extension is any with the guide RNA molecule except within the protospacer sequence, or at a position which disrupts the protospacer sequence.

[0615] In one embodiment, the intermolecular RNA extension is inserted downstream from the 3′ end of the protospacer sequence. In another embodiment, the intermolecular RNA extension is inserted at least 1 nucleotide, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12

nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides downstream of the 3′ end of the protospacer sequence.

[0616] In other embodiments, the intermolecular RNA extension is inserted into the gRNA, which refers to the portion of the guide RNA corresponding or comprising the tracrRNA, which binds and/or interacts with the Cas9 protein or equivalent thereof (i.e, a different napDNAbp). Preferably the insertion of the intermolecular RNA extension does not disrupt or minimally disrupts the interaction betweeen the tracrRNA portion and the napDNAbp.

[0617] The length of the RNA extension can be any useful length. In various embodiments, the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0618] The RT template sequence can also be any suitable length. For example, the RT template sequence can be at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0619] In still other embodiments, wherein the reverse transcription primer binding site sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18

nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0620] In other embodiments, the optional linker or spacer sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 30 nucleotides, at least 40 nucleotides, at least 50 nucleotides, at least 60 nucleotides, at least 70 nucleotides, at least 80 nucleotides, at least 90 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, at least 400 nucleotides, or at least 500 nucleotides in length.

[0621] The RT template sequence, in certain embodiments, encodes a single-stranded DNA molecule which is homologous to the non-target strand (and thus, complementary to the corresponding site of the target strand) but includes one or more nucleotide changes. The least one nucleotide change may include one or more single-base nucleotide changes, one or more deletions, and one or more insertions.

[0622] As depicted in FIG. 1G, the synthesized single-stranded DNA product of the RT template sequence is homologous to the non-target strand and contains one or more nucleotide changes. The single-stranded DNA product of the RT template sequence hybridizes in equilibrium with the complementary target strand sequence, thereby displacing the homologous endogenous target strand sequence. The displaced endogenous strand may be referred to in some embodiments as a 5′ endogenous DNA flap species (e.g., see FIG. 1E). This 5′ endogenous DNA flap species can be removed by a 5′ flap endonuclease (e.g., FEN1) and the single-stranded DNA product, now hybridized to the endogenous target strand, may be ligated, thereby creating a mismatch between the endogenous sequence and the newly synthesized strand. The mismatch may be resolved by the cell's innate DNA repair and/or replication processes.

[0623] In various embodiments, the nucleotide sequence of the RT template sequence corresponds to the nucleotide sequence of the non-target strand which becomes displaced as the 5′ flap species and which overlaps with the site to be edited.

[0624] In various embodiments of the extended guide RNAs, the reverse transcription template sequence may encode a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change. The single-stranded DNA flap may displace an endogenous single-strand DNA at the nick site. The displaced endogenous single-strand DNA at the nick site can have a 5′ end and form an endogenous flap, which can be excised by the cell. In various embodiments, excision of the 5′ end endogenous flap can help drive product formation since removing the 5′ end endogenous flap encourages hybridization of the single-strand 3′ DNA flap to the corresponding complementary DNA strand, and the incorporation or assimilation of the desired nucleotide change carried by the single-strand 3′ DNA flap into the target DNA.

[0625] In various embodiments of the extended guide RNAs, the cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[0626] In still other embodiments, the desired nucleotide change is installed in an editing window that is between about -5 to +5 of the nick site, or between about -10 to +10 of the nick site, or between about -20 to +20 of the nick site, or between about -30 to +30 of the nick site, or between about -40 to + 40 of the nick site, or between about -50 to +50 of the nick site, or between about -60 to +60 of the nick site, or between about -70 to +70 of the nick site, or between about -80 to +80 of the nick site, or between about -90 to +90 of the nick site, or between about -100 to +100 of the nick site, or between about -200 to +200 of the nick site.

In other embodiments, the desired nucleotide change is installed in an editing window that is between about +1 to +2 from the nick site, or about +1 to +3, +1 to +4, +1 to +5, +1 to +6, +1 to +7, +1 to +8, +1 to +9, +1 to +10, +1 to +11, +1 to +12, +1 to +13, +1 to +14, +1 to +15, +1 to +16, +1 to +17, +1 to +18, +1 to +19, +1 to +20, +1 to +21, +1 to +22, +1 to +23, +1 to +24, +1 to +25, +1 to +26, +1 to +27, +1 to +28, +1 to +29, +1 to +30, +1 to +31, +1 to +32, +1 to +33, +1 to +34, +1 to +35, +1 to +36, +1 to +37, +1 to +38, +1 to +39, +1 to +40, +1 to +41, +1 to +42, +1 to +43, +1 to +44, +1 to +45, +1 to +46, +1 to +47, +1 to +48, +1 to +49, +1 to +50, +1 to +51, +1 to +52, +1 to +53, +1 to +54, +1 to +55, +1 to +56, +1 to +57, +1 to +58, +1 to +59, +1 to +60, +1 to +61, +1 to +62, +1 to +63, +1 to +64, +1 to +65, +1 to +66, +1 to +67, +1 to +68, +1 to +69, +1 to +70, +1 to +71, +1 to +72, +1 to +73, +1 to +74, +1 to +75, +1 to +76, +1 to +77, +1 to +78, +1 to +79, +1 to +80, +1 to +81, +1 to +82, +1 to +83,

+1 to +84, +1 to +85, +1 to +86, +1 to +87, +1 to +88, +1 to +89, +1 to +90, +1 to +90, +1 to +91, +1 to +92, +1 to +93, +1 to +94, +1 to +95, +1 to +96, +1 to +97, +1 to +98, +1 to +99, +1 to +100, +1 to +101, +1 to +102, +1 to +103, +1 to +104, +1 to +105, +1 to +106, +1 to +107, +1 to +108, +1 to +109, +1 to +110, +1 to +111, +1 to +112, +1 to +113, +1 to +114, +1 to +115, +1 to +116, +1 to +117, +1 to +118, +1 to +119, +1 to +120, +1 to +121, +1 to +122, +1 to +123, +1 to +124, or +1 to +125 from the nick site.

[0627] In still other embodiments, the desired nucleotide change is installed in an editing window that is between about +1 to +2 from the nick site, or about +1 to +5, +1 to +10, +1 to +15, +1 to +20, +1 to +25, +1 to +30, +1 to +35, +1 to +40, +1 to +45, +1 to +50, +1 to +55, +1 to +100, +1 to +105, +1 to +110, +1 to +115, +1 to +120, +1 to +125, +1 to +130, +1 to +135, +1 to +140, +1 to +145, +1 to +150, +1 to +155, +1 to +160, +1 to +165, +1 to +170, +1 to +175, +1 to +180, +1 to +185, +1 to +190, +1 to +195, or +1 to +200, from the nick site.

[0628] In various aspects, the extended guide RNAs are modified versions of a guide RNA. Guide RNAs maybe naturally occurring, expressed from an encoding nucleic acid, or synthesized chemically. Methods are well known in the art for obtaining or otherwise synthesizing guide RNAs and for determining the appropriate sequence of the guide RNA, including the protospacer sequence which interacts and hybridizes with the target strand of a genomic target site of interest.

[0629] In various embodiments, the particular design aspects of a guide RNA sequence will depend upon the nucleotide sequence of a genomic target site of interest (i.e., the desired site to be edited) and the type of napDNAbp (e.g., Cas9 protein) present in prime editing systems described herein, among other factors, such as PAM sequence locations, percent G/C content in the target sequence, the degree of microhomology regions, secondary structures, etc.

[0630] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a napDNAbp (e.g., a Cas9, Cas9 homolog, or Cas9 variant) to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the

Burrows-Wheeler Transform (e.g., the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length.

[0631] In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of a prime editor (PE) to a target sequence may be assessed by any suitable assay. For example, the components of a prime editor (PE), including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of a prime editor (PE) disclosed herein, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a prime editor (PE), including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.

[0632] A guide sequence may be selected to target any target sequence. In some embodiments, the target sequence is a sequence within a genome of a cell. Exemplary target sequences include those that are unique in the target genome. For example, for the S. pyogenes Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMMNNNNNNNNNNNNXGG (SEQ ID NO: 204) where NNNNNNNNNNNNXGG (SEQ ID NO: 205) (N is A, G, T, or C; and X can be anything). A unique target sequence in a genome may include an S. pyogenes Cas9 target site of the form MMMMMMMMMNNNNNNNNNNNNXGG (SEQ ID NO: 206) where NNNNNNNNNNNXGG (SEQ ID NO: 207) (N is A, G, T, or C; and X can be anything). For the S. thermophilus CRISPR1Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMMNNNNNNNNNNNNXXAGAAW (SEQ ID NO: 208) where NNNNNNNNNNNNXXAGAAW (SEQ ID NO: 209) (N is A, G, T, or C; X can be anything; and W is A or T). A unique target sequence in a genome may include an S. thermophilus CRISPR 1 Cas9 target site of the form

MMMMMMMMMNNNNNNNNNNNXXAGAAW (SEQ ID NO: 210) where NNNNNNNNNNNXXAGAAW (SEQ ID NO: 211) (N is A, G, T, or C; X can be anything; and W is A or T). For the S. pyogenes Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMMMNNNNNNNNNNNXGGXG (SEQ ID NO: 212) where NNNNNNNNNNNXGGXG (SEQ ID NO: 213) (N is A, G, T, or C; and X can be anything). A unique target sequence in a genome may include an S. pyogenes Cas9 target site of the form MMMMMMMMMNNNNNNNNNNNXGGXG (SEQ ID NO: 214) where NNNNNNNNNNNXGGXG (SEQ ID NO: 215) (N is A, G, T, or C; and X can be anything). In each of these sequences "M" may be A, G, T, or C, and need not be considered in identifying a sequence as unique.

[0633] In some embodiments, a guide sequence is selected to reduce the degree of secondary structure within the guide sequence. Secondary structure may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is mFold, as described by Zuker and Stiegler (Nucleic Acids Res. 9 (1981), 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g. A. R. Gruber et al., 2008, Cell 106(1): 23-24; and PA Carr and GM Church, 2009, Nature Biotechnology 27(12): 1151-62). Further algorithms may be found in U.S. application Ser. No. 61/836,080; Broad Reference BI-2013/004A); incorporated herein by reference.

[0634] In general, a tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the corresponding tracr sequence; and (2) formation of a complex at a target sequence, wherein the complex comprises the tracr mate sequence hybridized to the tracr sequence. In general, degree of complementarity is with reference to the optimal alignment of the tracr mate sequence and tracr sequence, along the length of the shorter of the two sequences. Optimal alignment may be determined by any suitable alignment algorithm, and may further account for secondary structures, such as self-complementarity within either the tracr sequence or tracr mate sequence. In some embodiments, the degree of complementarity between the tracr sequence and tracr mate sequence along the length of the shorter of the two when optimally aligned is about or more than about 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99%, or higher. In some embodiments, the tracr sequence is about or more than about 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or more nucleotides in length. In some embodiments, the tracr sequence and tracr mate sequence are contained within a single transcript, such that hybridization between the two produces a transcript having a secondary structure, such as a hairpin. Preferred loop forming sequences for use in hairpin structures are four nucleotides in length, and most preferably have the sequence GAAA. However, longer or shorter loop sequences may be used, as may alternative sequences. The sequences preferably include a nucleotide triplet (for example, AAA), and an additional nucleotide (for example C or G). Examples of loop forming sequences include CAAA and AAAG. In an embodiment of the invention, the transcript or transcribed polynucleotide sequence has at least two or more hairpins. In preferred embodiments, the transcript has two, three, four or five hairpins. In a further embodiment of the invention, the transcript has at most five hairpins. In some embodiments, the single transcript further includes a transcription termination sequence; preferably this is a polyT sequence, for example six T nucleotides. Further non-limiting examples of single polynucleotides comprising a guide sequence, a tracr mate sequence, and a tracr sequence are as follows (listed 5′ to 3′), where "N" represents a base of a guide sequence, the first block of lower case letters represent the tracr mate sequence, and the second block of lower case letters represent the tracr sequence, and the final poly-T sequence represents the transcription terminator:

(1)NNNNNNNNGTTTTTGTACTCTCAAGATTTAGAAATAAATCTTGCAGAAGCTACA AAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTTTC GTTATTTAATTTTTT (SEQ ID NO: 216);

[0635] (2)NNNNNNNNNNNNNNNNNNGTTTTTGTACTCTCAGAAATGCAGAAGCTA CAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGTTT TCGTTATTTAATTTTTT (SEQ ID NO: 217);

[0636] (3)NNNNNNNNNNNNNNNNNNNNGTTTTTGTACTCTCAGAAATGCAGAAGC TACAAAGATAAGGCTTCATGCCGAAATCAACACCCTGTCATTTTATGGCAGGGTGT TTTTT (SEQ ID NO: 218);

[0637] (4)NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAA ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT (SEQ ID NO: 219);

[0638] (5)NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAA ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGTTTTTTT (SEQ ID NO: 220); AND

[0639] (6) NNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCATTTTTTTT (SEQ ID NO: 221).

[0640] In some embodiments, sequences (1) to (3) are used in combination with Cas9 from S. thermophilus CRISPR1. In some embodiments, sequences (4) to (6) are used in combination with Cas9 from S. pyogenes. In some embodiments, the tracr sequence is a separate transcript from a transcript comprising the tracr mate sequence.

[0641] It will be apparent to those of skill in the art that in order to target any of the fusion proteins comprising a Cas9 domain and a single-stranded DNA binding protein, as disclosed herein, to a target site, e.g., a site comprising a point mutation to be edited, it is typically necessary to co-express the fusion protein together with a guide RNA, e.g., an sgRNA. As explained in more detail elsewhere herein, a guide RNA typically comprises a tracrRNA framework allowing for Cas9 binding, and a guide sequence, which confers sequence specificity to the Cas9:nucleic acid editing enzyme/domain fusion protein.

[0642] In some embodiments, the guide RNA comprises a structure 5′-[guide sequence]-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUU-3′ (SEQ ID NO: 222), wherein the guide sequence comprises a sequence that is complementary to the target sequence. The guide sequence is typically 20 nucleotides long. The sequences of suitable guide RNAs for targeting Cas9:nucleic acid editing enzyme/domain fusion proteins to specific genomic target sites will be apparent to those of skill in the art based on the instant disclosure. Such suitable guide RNA sequences typically comprise guide sequences that are complementary to a nucleic sequence within 50 nucleotides upstream or downstream of the target nucleotide to be edited. Some exemplary guide RNA sequences suitable for targeting any of the provided fusion proteins to specific target sequences are provided herein. Additional guide sequences are well known in the art and can be used with the prime editor (PE) described herein.

[0643] In other embodiments, the PEgRNAs include those depicted in FIG. 3D.

[0644] In still other embodiments, the PEgRNAs may include those depicted in FIG. 3E.

[0645] FIG. 3D provides the structure of an embodiment of a PEgRNA contemplated herein and which may be designed in accordance with the methodology defined in Example 2. The PEgRNA comprises three main component elements ordered in the 5′ to 3′ direction, namely: a spacer, a gRNA core, and an extension arm at the 3′ end. The extension arm may further be divided into the following structural elements in the 5′ to 3′ direction, namely: a primer

binding site (A), an edit template (B), and a homology arm (C). In addition, the PEgRNA may comprise an optional 3′ end modifier region (e1) and an optional 5′ end modifier region (e2). Still further, the PEgRNA may comprise a transcriptional termination signal at the 3′ end of the PEgRNA (not depicted). These structural elements are further defined herein. The depiction of the structure of the PEgRNA is not meant to be limiting and embraces variations in the arrangement of the elements. For example, the optional sequence modifiers (e1) and (e2) could be positioned within or between any of the other regions shown, and not limited to being located at the 3′ and 5′ ends.

[0646] FIG. 3E provides the structure of another embodiment of a PEgRNA contemplated herein and which may be designed in accordance with the methodology defined in Example 2. The PEgRNA comprises three main component elements ordered in the 5′ to 3′ direction, namely: a spacer, a gRNA core, and an extension arm at the 3′ end. The extension arm may further be divided into the following structural elements in the 5′ to 3′ direction, namely: a primer binding site (A), an edit template (B), and a homology arm (C). In addition, the PEgRNA may comprise an optional 3′ end modifier region (e1) and an optional 5′ end modifier region (e2). Still further, the PEgRNA may comprise a transcriptional termination signal on the 3′ end of the PEgRNA (not depicted). These structural elements are further defined herein. The depiction of the structure of the PEgRNA is not meant to be limiting and embraces variations in the arrangement of the elements. For example, the optional sequence modifiers (e1) and (e2) could be positioned within or between any of the other regions shown, and not limited to being located at the 3′ and 5′ ends.

PEgRNA improvements

[0647] The PEgRNAs may also include additional design improvements that may modify the properties and/or characteristics of PEgRNAs thereby improving the efficacy of prime editing. In various embodiments, these improvements may belong to one or more of a number of different categories, including but not limited to: (1) designs to enable efficient expression of functional PEgRNAs from non-polymerase III (pol III) promoters, which would enable the expression of longer PEgRNAs without burdensome sequence requirements; (2) improvements to the core, Cas9-binding PEgRNA scaffold, which could improve efficacy; (3) modifications to the PEgRNA to improve RT processivity, enabling the insertion of longer sequences at targeted genomic loci; and (4) addition of RNA motifs to the 5′ or 3′ termini of the PEgRNA that improve PEgRNA stability, enhance RT processivity,

prevent misfolding of the PEgRNA, or recruit additional factors important for genome editing.

[0648] In one embodiment, PEgRNA could be designed with polIII promoters to improve the expression of longer-length PEgRNA with larger extension arms. sgRNAs are typically expressed from the U6 snRNA promoter. This promoter recruits pol III to express the associated RNA and is useful for expression of short RNAs that are retained within the nucleus. However, pol III is not highly processive and is unable to express RNAs longer than a few hundred nucleotides in length at the levels required for efficient genome editing. Additionally, pol III can stall or terminate at stretches of U's, potentially limiting the sequence diversity that could be inserted using a PEgRNA. Other promoters that recruit polymerase II (such as pCMV) or polymerase I (such as the U1 snRNA promoter) have been examined for their ability to express longer sgRNAs. However, these promoters are typically partially transcribed, which would result in extra sequence 5′ of the spacer in the expressed PEgRNA, which has been shown to result in markedly reduced Cas9:sgRNA activity in a site-dependent manner. Additionally, while pol III-transcribed PEgRNAs can simply terminate in a run of 6-7 U's, PEgRNAs transcribed from pol II or pol I would require a different termination signal. Often such signals also result in polyadenylation, which would result in undesired transport of the PEgRNA from the nucleus. Similarly, RNAs expressed from pol II promoters such as pCMV are typically 5′-capped, also resulting in their nuclear export.

[0649] Previously, Rinn and coworkers screened a variety of expression platforms for the production of long-noncoding RNA- (lncRNA) tagged sgRNAs[183]. These platforms include RNAs expressed from pCMV and that terminate in the ENE element from the MALAT1 ncRNA from humans[184], the PAN ENE element from KSHV[185], or the 3′ box from U1 snRNA[186]. Notably, the MALAT1 ncRNA and PAN ENEs form triple helices protecting the polyA-tail [184, 187]. These constructs could also enhance RNA stability. It is contemplated that these expression systems will also enable the expression of longer PEgRNAs.

[0650] In addition, a series of methods have been designed for the cleavage of the portion of the pol II promoter that would be transcribed as part of the PEgRNA, adding either a self-cleaving ribozyme such as the hammerhead[188], pistol[189], hatchet[189], hairpin[190], VS[191], twister[192], or twister sister[192] ribozymes, or other self-cleaving elements to process the transcribed guide, or a hairpin that is recognized by Csy4[193] and also leads to processing of the guide. Also, it is hypothesized that incorporation of multiple ENE motifs could lead to

improved PEgRNA expression and stability, as previously demonstrated for the KSHV PAN RNA and element[185]. It is also anticipated that circularizing the PEgRNA in the form of a circular intronic RNA (ciRNA) could also lead to enhanced RNA expression and stability, as well as nuclear localization[194].

[0651] In various embodiments, the PEgRNA may include various above elements, as exemplified by the following sequence.

[0652] Non-limiting example 1 - PEgRNA expression platform consisting of pCMV, Csy4 hairpin, the PEgRNA, and MALAT1 ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTAGGGTCATGAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTT
CTCAGGTTTTGCTTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGCAAAAGAT
GCTGGTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAATCCCTGCGGCGTCT
TTGCTTTGACT (SEQ ID NO: 223)

[0653] Non-limiting example 2 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and PAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC

ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAA (SEQ ID NO: 224)

[0654] Non-limiting example 3 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and 3xPAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC
ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAACACACTGT
TTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAG
GTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGC
CTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAA
GGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAATCTCTCTGTTTTGGCTGG
GTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAGGTTTTTATCC
CAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGCCTAGAGCTCA
AATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAAGGTCAATGTTT
AATCCATAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 225)

[0655] Non-limiting example 4 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and 3′ box

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTTGTTTGGTT
TGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 226)

[0656] Non-limiting example 5 - PEgRNA expression platform consisting of pU1, Csy4 hairping, the PEgRNA, and 3′ box

CTAAGGACCAGCTTCTTTGGGAGAGAACAGACGCAGGGGCGGGAGGGAAAAAG
GGAGAGGCAGACGTCACTTCCCCTTGGCGGCTCTGGCAGCAGATTGGTCGGTTGA
GTGGCAGAAAGGCAGACGGGGACTGGGCAAGGCACTGTCGGTGACATCACGGAC
AGGGCGACTTCTATGTAGATGAGGCAGCGCAGAGGCTGCTGCTTCGCCACTTGCT
GCTTCACCACGAAGGAGTTCCCGTGCCCTGGGAGCGGGTTCAGGACCGCTGATCG
GAAGTGAGAATCCCAGCTGTGTGTCAGGGCTGGAAAGGGCTCGGGAGTGCGCGG
GGCAAGTGACCGTGTGTGTAAAGAGTGAGGCGTATGAGGCTGTGTCGGGGCAGA
GGCCCAAGATCTCAGTTCACTGCCGTATAGGCAGGGCCCAGACTGAGCACGTGAG
TTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAGTCTGTTTCAGCAAG
TTCAGAGAAATCTGAACTTGCTGGATTTTTGGAGCAGGGAGATGGAATAGGAGCT
TGCTCCGTCCACTCCACGCATCGACCTGGTATTGCAGTACCTCCAGGAACGGTGC
ACCCACTTTCTGGAGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTT
GTTTGGTTTGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 227).

[0657] In various other embodiments, the PEgRNA may be improved by introducing improvements to the scaffold or core sequences. This can be done by introducing known

[0658] The core, Cas9-binding PEgRNA scaffold can likely be improved to enhance PE activity. Several such approaches have already been demonstrated. For instance, the first pairing element of the scaffold (P1) contains a GTTTT-AAAAC pairing element. Such runs of Ts have been shown to result in pol III pausing and premature termination of the RNA transcript. Rational mutation of one of the T-A pairs to a G-C pair in this portion of P1 has been shown to enhance sgRNA activity, suggesting this approach would also be feasible for PEgRNAs[195]. Additionally, increasing the length of P1 has also been shown to enhance sgRNA folding and lead to improved activity[195], suggesting it as another avenue for the improvement of PEgRNA activity. Example improvements to the core can include:

[0659] PEgRNA containing a 6 nt extension to P1

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGCTCATGAAAATGAGCTAGCAAG
TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTC
TGCCATCAAAGCGTGCTCAGTCTGTTTTTTT (SEQ ID NO: 228)

[0660] PEgRNA containing a T-A to G-C mutation within P1

GGCCCAGACTGAGCACGTGAGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGTTTTTTT (SEQ ID NO: 229)

[0661] In various other embodiments, the PEgRNA may be improved by introducing modifications to the edit template region. As the size of the insertion templated by the PEgRNA increases, it is more likely to be degraded by endonucleases, undergo spontaneous

hydrolysis, or fold into secondary structures unable to be reverse-transcribed by the RT or that disrupt folding of the PEgRNA scaffold and subsequent Cas9-RT binding. Accordingly, it is likely that modification to the template of the PEgRNA might be necessary to affect large insertions, such as the insertion of whole genes. Some strategies to do so include the incorporation of modified nucleotides within a synthetic or semi-synthetic PEgRNA that render the RNA more resistant to degradation or hydrolysis or less likely to adopt inhibitory secondary structures[196]. Such modifications could include 8-aza-7-deazaguanosine, which would reduce RNA secondary structure in G-rich sequences; locked-nucleic acids (LNA) that reduce degradation and enhance certain kinds of RNA secondary structure; 2'-O-methyl, 2'-fluoro, or 2'-O-methoxyethoxy modifications that enhance RNA stability. Such modifications could also be included elsewhere in the PEgRNA to enhance stability and activity. Alternatively or additionally, the template of the PEgRNA could be designed such that it both encodes for a desired protein product and is also more likely to adopt simple secondary structures that are able to be unfolded by the RT. Such simple structures would act as a thermodynamic sink, making it less likely that more complicated structures that would prevent reverse transcription would occur. Finally, one could also split the template into two, separate PEgRNAs. In such a design, a PE would be used to initiate transcription and also recruit a separate template RNA to the targeted site via an RNA-binding protein fused to Cas9 or an RNA recognition element on the PEgRNA itself such as the MS2 aptamer. The RT could either directly bind to this separate template RNA, or initiate reverse transcription on the original PEgRNA before swapping to the second template. Such an approach could enable long insertions by both preventing misfolding of the PEgRNA upon addition of the long template and also by not requiring dissociation of Cas9 from the genome for long insertions to occur, which could possibly be inhibiting PE-based long insertions.

[0662] In still other embodiments, the PEgRNA may be improved by introducing additional RNA motifs at the 5′ and 3′ termini of the PEgRNAs, or even at positions therein between (e.g., in the gRNA core region, or the the spacer). Several such motifs - such as the PAN ENE from KSHV and the ENE from MALAT1 were discussed above as possible means to terminate expression of longer PEgRNAs from non-pol III promoters. These elements form RNA triple helices that engulf the polyA tail, resulting in their being retained within the nucleus[184, 187]. However, by forming complex structures at the 3′ terminus of the PEgRNA that occlude the terminal nucleotide, these structures would also likely help prevent exonuclease-mediated degradation of PEgRNAs.

[0663] Other structural elements inserted at the 3′ terminus could also enhance RNA stability, albeit without enabling termination from non-pol III promoters. Such motifs could include hairpins or RNA quadruplexes that would occlude the 3′ terminus[197], or self-cleaving ribozymes such as HDV that would result in the formation of a 2'-3′-cyclic phosphate at the 3′ terminus and also potentially render the PEgRNA less likely to be degraded by exonucleases[198]. Inducing the PEgRNA to cyclize via incomplete splicing - to form a ciRNA - could also increase PEgRNA stability and result in the PEgRNA being retained within the nucleus[194].

[0664] Additional RNA motifs could also improve RT processivity or enhance PEgRNA activity by enhancing RT binding to the DNA-RNA duplex. Addition of the native sequence bound by the RT in its cognate retroviral genome could enhance RT activity[199]. This could include the native primer binding site (PBS), polypurine tract (PPT), or kissing loops involved in retroviral genome dimerization and initiation of transcription[199].

[0665] Addition of dimerization motifs - such as kissing loops or a GNRA tetraloop/tetraloop receptor pair[200] - at the 5′ and 3′ termini of the PEgRNA could also result in effective circularization of the PEgRNA, improving stability. Additionally, it is envisioned that addition of these motifs could enable the physical separation of the PEgRNA spacer and primer, prevention occlusion of the spacer which would hinder PE activity. Short 5′ extensions or 3' extensions to the PEgRNA that form a small toehold hairpin in the spacer region or along the primer binding site could also compete favorably against the annealing of intracomplementary regions along the length of the PEgRNA, e.g., the interaction between the spacer and the primer binding site that can occur.Finally, kissing loops could also be used to recruit other template RNAs to the genomic site and enable swapping of RT activity from one RNA to the other. As exemplary embodiments of various secondary structures, the PEgRNA depicted in FIG. 3D and FIG. 3E list a number secondary RNA structures that may be engineered into any region of the PEgRNA, including in the terminal portions of the extension arm (i.e., e1and e2), as shown.

[0666] Example improvements include, but are not limited to:

[0667] PEgRNA-HDV fusion

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAA
CATGCTTCGGCATGGCGAATGGGACTTTTTTT (SEQ ID NO: 230)

[0668] PEgRNA-MMLV kissing loop

GGTGGGAGACGTCCCACCGGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAA
TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTC
GGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACC
TTTTTTT (SEQ ID NO: 231)

[0669] PEgRNA-VS ribozyme kissing loop

GAGCAGCATGGCGTCGCTGCTCACGGCCCAGACTGAGCACGTGAGTTTTAGAGCT
AGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACC
GAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTCCATCAGTTGACA
CCCTGAGGTTTTTTT (SEQ ID NO: 232)

[0670] PEgRNA-GNRA tetraloop/tetraloop receptor

GCAGACCTAAGTGGUGACATATGGTCTGGGCCCAGACTGAGCACGTGAGTTTTAG
AGCTAUACGTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTUACGAAGTGG
GACCGAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGCATGCGATT
AGAAATAATCGCATGTTTTTTT (SEQ ID NO: 233)

[0671] PEgRNA template switching secondary RNA-HDV fusion

TCTGCCATCAAAGCTGCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACCGGCCG
GCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG
AATGGGACTTTTTTT (SEQ ID NO: 234)

[0672] PEgRNA scaffold could be further improved via directed evolution, in an analogous fashion to how SpCas9 and prime editor (PE) have been improved. Directed evolution could enhance PEgRNA recognition by Cas9 or evolved Cas9 variants. Additionally, it is likely that different PEgRNA scaffold sequences would be optimal at different genomic loci, either enhancing PE activity at the site in question, reducing off-target activities, or both. Finally, evolution of PEgRNA scaffolds to which other RNA motifs have been added would almost certainly improve the activity of the fused PEgRNA relative to the unevolved, fusion RNA. For instance, evolution of allosteric ribozymes composed of c-di-GMP-I aptamers and hammerhead ribozymes led to dramatically improved activity[202], suggesting that evolution would improve the activity of hammerhead-PEgRNA fusions as well. In addition, while Cas9 currently does not generally tolerate 5′ extension of the sgRNA, directed evolution will likely generate enabling mutations that mitigate this intolerance, allowing additional RNA motifs to be utilized.

[0673] The present disclosure contemplates any such ways to further improve the efficacy of the prime editing systems disclosed here.

[0674] In various embodiments, it may be advantageous to limit the appearance of consecutive sequence of Ts from the extension arm as consecutive series of T's may limit the capacity of the PEgRNA to be transcribed. For example, strings of at least consecutive three T's, at least consecutive four T's, at least consecutive five T's, at least consecutive six T's, at

least consecutive seven T's, at least consecutive eight T's, at least consecutive nine T's, at least consecutive ten T's, at least consecutive elevent T's, at least consecutive twelve T's, at least consecutive thirteen T's , at least consecutive fourteen T's, or at least consecutive fifteen T's should be avoided when designing the PEgRNA, or should be at least removed from the final designed sequence. In one embodiment, one can avoid the includes of unwanted strings of consecutive T's in PEgRNA extension arms but avoiding target sites that are rich in consecutive A:T nucleobase pairs.

Split PEgRNA designs for *trans* prime editing

[0675] The instant disclosure also contemplates *trans* prime editing, which refers to a modified version of prime editing which operates by separating the PEgRNA into two distinct molecules: a guide RNA and a tPERT molecule. The tPERT molecule is programmed to co-localize with the prime editor complex at a target DNA site, bringing the primer binding site and the DNA synthesis template to the prime editor *in trans*. For example, see FIG. 3G for an embodiment of a *trans* prime editor (tPE) which shows a two-component system comprising (1) an recruiting protein (RP)-PE:gRNA complex and (2) a tPERT that includes a primer binding site and a DNA synthesis template joined to an RNA-protein recruitment domain (e.g., stem loop or hairpin), wherein the recruiting protein component of the RP-PE:gRNA complex recruits the tPERT to a target site to be edited, thereby associating the PBS and DNA synthesis template with the prime editor *in trans*. Said another way, the tPERT is engineered to contain (all or part of) the extension arm of a PEgRNA, which includes the primer binding site and the DNA synthesis template. One advantage of this approach is to separate the extension arm of a PEgRNA from the guide RNA, thereby minimizing annealing interactions that tend to occur between the PBS of the extension arm and the spacer sequence of the guide RNA.

[0676] A key feature of *trans* prime editing is the ability of the *trans* prime editor to recruit the tPERT to the site of DNA editing, thereby effectively co-localizing all of the functions of a PEgRNA at the site of prime editing. Recruitment can be achieve by installing an RNA-protein recruitment domain, such as a MS2 aptamer, into the tPERT and fusing a corresponding recruiting protein to the prime editor (e.g., via a linker to the napDNAbp or via a linker to the polymerase) that is capable of specifically binding to the RNA-protein recruitment domain, thereby recruiting the tPERT molecule to the prime editor complex. As depicted in the process described in FIG. 3H, the RP-PE:gRNA complex binds to and nicks the target DNA sequence. Then, the recruiting protein (RP) recruits a tPERT to co-localize to

the prime editor complex bound to the DNA target site, thereby allowing the primer binding site, located on the tPERT, to bind to the primer sequence on the nicked strand, and subsequently, allowing the polymerase (e.g., RT) to synthesize a single strand of DNA against the DNA synthesis template, located on the tPERT, up through the 5′ end of the tPERT.

[0677] While the tPERT is shown in FIG. 3G and FIG. 3H as comprising the PBS and DNA synthesis template on the 5′ end of the RNA-protein recruitment domain, the tPERT in other configurations may be designed with the PBS and DNA synthesis template located on the 3′ end of the RNA-protein recruitment domain. However, the tPERT with the 5' extension has the advantage that synthesis of the single strand of DNA will naturally terminate at the 5' end of the tPERT and thus, does not risk using any portion of the RNA-protein recruitment domain as a template during the DNA synthesis stage of prime editing.

PEgRNA design method

[0678] The present disclosure also relates to methods for designing PEgRNAs.

[0679] In one aspect of design, the design approach can take into account the particular application for which prime editing is being used. For instance, and as exemplied and discussed herein, prime editing can be used, without limitation, to (a) install mutation-correcting changes to a nucleotide sequence, (b) install protein and RNA tags, (c) install immunoepitopes on proteins of interest, (d) install inducible dimerization domains in proteins, (e) install or remove sequences to alter that activity of a biomolecule, (f) install recombinase target sites to direct specific genetic changes, and (g) mutagenesis of a target sequence by using an error-prone RT. In addition to these methods which, in general, insert, change, or delete nucleotide sequences at target sites of interest, prime editors can also be used to construct highly programmable libraries, as well as to conduct cell data recording and lineage tracing studies. In these various uses, there may be as described herein particular design aspects pertaining to the preparation of a PEgRNA that is particularly useful for any given of these applications.

[0680] When designing a PEgRNA for any particular application or use of prime editing, a number of considerations may be taken into account, which include, but are not limited to:

(a) the target sequence, i.e., the nucleotide sequence in which one or more nucleobase modifications are desired to be installed by the prime editor;

(b) the location of the cut site within the target sequence, i.e., the specific nucleobase position at which the prime editor will induce a single-stand nick to create a 3′ end RT primer

sequence on one side of the nick and the 5′ end endogenous flap on the other side of the nick (which ultimately is removed by FEN1 or equivalent thereto and replaced by the 3′ ssDNA flap. The cut site is analogous to the "edit location" since this what creates the 3′ end RT primer sequence which becomes extended by the RT during RNA-depending DNA polymerization to create the 3′ ssDNA flap containing the desired edit, which then replaces the 5′ endogenous DNA flap in the target sequence.

(c) the available PAM sequences (including the canonical SpCas9 PAM sites, as well as non-canonical PAM sites recognized by Cas9 variants and equivalents with expanded or differing PAM specificities);

(d) the spacing between the available PAM sequences and the location of the cut site in the target sequence;

(e) the particular Cas9, Cas9 variant, or Cas9 equivalent of the prime editor being used;

(f) the sequence and length of the primer binding site;

(g) the sequence and length of the edit template;

(h) the sequence and length of the homology arm;

(i) the spacer sequence and length; and

(j) the core sequence.

[0681] The instant disclosure discusses these aspects above.

[0682] In one embodiment, an approach to designing a suitable PEgRNA, and optionally a nicking-sgRNA design guide for second-site nicking, is hereby provided. This embodiment provides a step-by-step set of instructions for designing PEgRNAs and nicking-sgRNAs for prime editing which takes into account one or more of the above considerations. The steps reference the examples shown in FIGs. 70A-70I.

1. **Define the target sequence and the edit**. Retrieve the sequence of the target DNA region (~200bp) centered around the location of the desired edit (point mutation, insertion, deletion, or combination thereof). See FIG. 70A.
2. **Locate target PAMs**. Identify PAMs in the proximity to the desired edit location. PAMs can be identified on either strand of DNA proximal to the desired edit location. While PAMs close to the edit position are preferred (i.e., wherein the nick site is less than 30 nt from the edit position, or less than 29 nt, 28 nt, 27 nt, 26 nt, 25 nt, 24 nt, 23 nt, 22 nt, 21 nt, 20 nt, 19 nt, 18 nt, 17 nt, 16 nt, 15 nt, 14 nt, 13 nt, 12 nt, 11 nt, 10 nt, 9 nt, 8 nt, 7 nt, 6 nt, 5 nt, 4 nt, 3 nt, or 2 nt from the edit position to the nick site), it is

possible to install edits using protospacers and PAMs that place the nick ≥ 30 nt from the edit position. See FIG. 70B.

3. **Locate the nick sites**. For each PAM being considered, identify the corresponding nick site and on which strand. For Sp Cas9 H840A nickase, cleavage occurs in the PAM-containing strand between the 3rd and 4th bases 5′ to the NGG PAM. All edited nucleotides must exist 3′ of the nick site, so appropriate PAMs must place the nick 5′ to the target edit on the PAM-containing strand. In the example shown below, there are two possible PAMs. For simplicity, the remaining steps will demonstrate the design of a PEgRNA using PAM 1 only. See FIG. 70C.
4. **Design the spacer sequence**. The protospacer of Sp Cas9 corresponds to the 20 nucleotides 5′ to the NGG PAM on the PAM-containing strand. Efficient Pol III transcription initiation requires a G to be the first transcribed nucleotide. If the first nucleotide of the protospacer is a G, the spacer sequence for the PEgRNA is simply the protospacer sequence. If the first nucleotide of the protospacer is not a G, the spacer sequence of the PEgRNA is G followed by the protospacer sequence. See FIG. 70D.
5. **Design a primer binding site (PBS)**. Using the starting allele sequence, identify the DNA primer on the PAM-containing strand. The 3′ end of the DNA primer is the nucleotide just upstream of the nick site (i.e. the 4th base 5′ to the NGG PAM for Sp Cas9). As a general design principle for use with PE2 and PE3, a PEgRNA primer binding site (PBS) containing 12 to 13 nucleotides of complementarity to the DNA primer can be used for sequences that contain ~40-60% GC content. For sequences with low GC content, longer (14- to 15-nt) PBSs should be tested. For sequences with higher GC content, shorter (8- to 11-nt) PBSs should be tested. Optimal PBS sequences should be determined empirically, regardless of GC content. To design a length-*p* PBS sequence, take the reverse complement of the first *p* nucleotides 5′ of the nick site in the PAM-containing strand using the *starting* allele sequence. See FIG. 70E.
6. **Design an RT template (or DNA synthesis template)**. The RT template (or DNA synthesis template where the polymerase is not reverse transcriptase) encodes the designed edit and homology to the sequence adjacent to the edit. In one embodiment, these regions correspond to the DNA synthesis template of FIG. 3D and FIG. 3E, wherein the DNA synthesis template comprises the "edit template" and the

"homology arm." Optimal RT template lengths vary based on the target site. For short-range edits (positions +1 to +6), it is recommended to test a short (9 to 12 nt), a medium (13 to 16 nt), and a long (17 to 20 nt) RT template. For long-range edits (positions +7 and beyond), it is recommended to use RT templates that extend at least 5 nt (preferably 10 or more nt) past the position of the edit to allow for sufficient 3′ DNA flap homology. For long-range edits, several RT templates should be screened to identify functional designs. For larger insertions and deletions (≥5 nt), incorporation of greater 3′ homology (~20 nt or more) into the RT template is recommended. Editing efficiency is typically impaired when the RT template encodes the synthesis of a G as the last nucleotide in the reverse transcribed DNA product (corresponding to a C in the RT template of the PEgRNA). As many RT templates support efficient prime editing, avoidance of G as the final synthesized nucleotide is recommended when designing RT templates. To design a length-*r* RT template sequence, use the *desired* allele sequence and take the reverse complement of the first *r* nucleotides 3′ of the nick site in the strand that originally contained the PAM. Note that compared to SNP edits, insertion or deletion edits using RT templates of the same length will not contain identical homology. See FIG. 70F.

7. **Assemble the full PEgRNA sequence**. Concatenate the PEgRNA components in the following order (5′ to 3′): spacer, scaffold, RT template and PBS. See FIG. 70G.
8. **Designing nicking-sgRNAs for PE3**. Identify PAMs on the non-edited strand upstream and downstream of the edit. Optimal nicking positions are highly locus-dependent and should be determined empirically. In general, nicks placed 40 to 90 nucleotides 5′ to the position across from the PEgRNA-induced nick lead to higher editing yields and fewer indels. A nicking sgRNA has a spacer sequence that matches the 20-nt protospacer in the *starting* allele, with the addition of a 5′-G if the protospacer does not begin with a G. See FIG. 70H.
9. **Designing PE3b nicking-sgRNAs**. If a PAM exists in the complementary strand and its corresponding protospacer overlaps with the sequence targeted for editing, this edit could be a candidate for the PE3b system. In the PE3b system, the spacer sequence of the nicking-sgRNA matches the sequence of the desired edited allele, but not the starting allele. The PE3b system operates efficiently when the edited nucleotide(s) falls within the seed region (~10 nt adjacent to the PAM) of the nicking-sgRNA protospacer. This prevents nicking of the complementary strand until after installation

of the edited strand, preventing competition between the PEgRNA and the sgRNA for binding the target DNA. PE3b also avoids the generation of simultaneous nicks on both strands, thus reducing indel formation significantly while maintaining high editing efficiency. PE3b sgRNAs should have a spacer sequence that matches the 20-nt protospacer in the *desired* allele, with the addition of a 5′ G if needed. See FIG. 70I.

[0683] The above step-by-step process for designing a suitable PEgRNA and a second-site nicking sgRNA is not meant to be limiting in any way. The disclosure contemplates variations of the above-described step-by-step process which would be derivable therefrom by a person of ordinary skill in the art.

[7] Applications utilizing prime editing

[0684] In addition to the development of the prime editing system described herein as a new "search-and-replace" genome editing technology that mediates targeted insertions, deletions, and all 12 possible base-to-base conversions at targeted loci in human cells without requiring double-stranded DNA breaks, or donor DNA templates, the inventors have also contemplated the use of the prime editors in a wide-array of specific applications. For example, and as exemplied and discussed herein, prime editing can be used to (a) install mutation-correcting changes to a nucleotide sequence, (b) install protein and RNA tags, (c) intallation of immunoepitopes on proteins of interest, (d) install inducible dimerization domains in proteins, (e) install or remove sequences to alter that activity of a biomolecule, (f) install recombinase target sites to direct specific genetic changes, and (g) mutagenesis of a target sequence by using an error-prone RT. In addition to these methods which, in general, insert, change, or delete nucleotide sequences at target sites of interest, prime editors can also be used to construct highly programmable libraries, as well as to conduct cell data recording and lineage tracing studies. The inventors have also contemplated additional design features of PEgRNAs that are aimed to improve the efficacy of prime editing. Still further, the inventors have conceived of methods for successfully delivering prime editors using vector delivery systems and which involve splitting the napDNAbp using intein domains.

[0685] These specific exemplary uses of prime editing are in no way intended to be limiting. The present Application contemplates any use for prime editing which involves, in general, some form of the installation, removal, and/or modification of one or more nucleobases at a target site in a nucleotide sequence, e.g., a genomic DNA.

[0686] For any of the exemplified uses for prime editing, one may use any prime editor disclosed herein, including PE1, PE2, PE3, and PE3b, or PE-short.

A. Prime editing mechanism

[0687] In various embodiments, prime editing (or "prime editing") operates by contacting a target DNA molecule (for which a change in the nucleotide sequence is desired to be introduced) with a nucleic acid programmable DNA binding protein (napDNAbp) complexed with an extended guide RNA. In reference to FIG. 1G, the extended guide RNA comprises an extension at the 3′ or 5′ end of the guide RNA, or at an intramolecular location in the guide RNA and encodes the desired nucleotide change (e.g., single nucleotide change, insertion, or deletion). In step (a), the napDNAbp/extended gRNA complex contacts the DNA molecule and the extended gRNA guides the napDNAbp to bind to a target locus. In step (b), a nick in one of the strands of DNA of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand, i.e., the strand that is not hybridized to the guide RNA sequence, i.e., the "non-target strand." The nick, however, could be introduced in either of the strands. That is, the nick could be introduced into the R-loop "target strand" (i.e., the strand hybridized to the protospacer sequence of the extended gRNA) or the "non-target strand" (i.e, the strand forming the single-stranded portion of the R-loop and which is complementary to the target strand). In step (c), the 3′ end of the DNA strand (formed by the nick) interacts with the extended portion of the guide RNA in order to prime reverse transcription (i.e, "target-primed RT"). In certain embodiments, the 3′ end DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA, i.e, the "reverse transcriptase priming sequence." In step (d), a reverse transcriptase is introduced (as a fusion protein with the napDNAbp or *in trans*) which synthesizes a single strand of DNA from the 3′ end of the primed site towards the 5′ end of the extended guide RNA. This forms a single-strand DNA flap comprising the desired nucleotide change (e.g., the single base change, insertion, or deletion, or a combination thereof) and which is otherwise homologous to the endogenous DNA at or adjacent to the nick site. In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap such that the desired nucleotide change becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap (e.g., by FEN1 or similar enzyme that is provide

in trans, as a fusion with the prime editor, or endogenously provided) that forms once the 3′ single strand DNA flap invades and hybridizes to the endogenous DNA sequence. Without being bound by theory, the cells endogenous DNA repair and replication processes resolves the mismatched DNA to incorporate the nucleotide change(s) to form the desired altered product. The process can also be driven towards product formation with "second strand nicking," as exemplified in FIG. 1G, or "termporal second strand nicking," as exemplified in FIG. 1I and discussed herein.

[0688] The process of prime editing may introduce at least one or more of the following genetic changes: transversions, transitions, deletions, and insertions. In addition, prime editing may be implemented for specific applications. For example, and as exemplied and discussed herein, prime editing can be used to (a) install mutation-correcting changes to a nucleotide sequence, (b) install protein and RNA tags, (c) intallation of immunoepitopes on proteins of interest, (d) install inducible dimerization domains in proteins, (e) install or remove sequences to alter that activity of a biomolecule, (f) install recombinase target sites to direct specific genetic changes, and (g) mutagenesis of a target sequence by using an error-prone RT. In addition to these methods which, in general, insert, change, or delete nucleotide sequences at target sites of interest, prime editors can also be used to construct highly programmable libraries, as well as to conduct cell data recording and lineage tracing studies. The inventors have also contemplated additional design features of PEgRNAs that are aimed to improve the efficacy of prime editing. Still further, the inventors have conceived of methods for successfully delivering prime editors using vector delivery systems and which involve splitting the napDNAbp using intein domains.

[0689] The term "prime editing system" or "prime editor (PE)" refers the compositions involved in the method of genome editing using target-primed reverse transcription (TPRT) describe herein, including, but not limited to the napDNAbps, reverse transcriptases, fusion proteins (e.g., comprising napDNAbps and reverse transcriptases), extended guide RNAs, and complexes comprising fusion proteins and extended guide RNAs, as well as accessory elements, such as second strand nicking components and 5′ endogenous DNA flap removal endonucleases (e.g., FEN1) for helping to drive the prime editing process towards the edited product formation.

[0690] In another embodiment, the schematic of FIG. 3F depicts the interaction of a typical PEgRNA with a target site of a double stranded DNA and the concomitant production of a 3′ single stranded DNA flap containing the genetic change of interest. The double strand DNA

is shown with the top strand in the 3′ to 5′ orientation and the lower strand in the 5′ to 3′ direction. The top strand comprises the "protospacer" and the PAM sequence and is referred to as the "target strand." The complementary lower strand is referred to as the "non-target strand." Although not shown, the PEgRNA depicted would be complexed with a Cas9 or equivalent. As shown in the schematic, the spacer of the PEgRNA anneals to a complementary region on the target strand, which is referred to as the protospacer, which is located just downstream of the PAM sequence is approximately 20 nucleotides in length. This interaction forms as DNA/RNA hybrid between the spacer RNA and the protospacer DNA, and induces the formation of an R loop in the region opposite the protospacer. As taught elsewhere herein, the Cas9 protein (not shown) then induces a nick in the non-target strand, as shown. This then leads to the formation of the 3′ ssDNA flap region which, in accordance with *z*, interacts with the 3′ end of the PEgRNA at the primer binding site. The 3′ end of the ssDNA flap (i.e., the reverse transcriptase primer sequence) anneals to the primer binding site (A) on the PEgRNA, thereby priming reverse transcriptase. Next, reverse transcriptase (e.g., provided *in trans* or provided *cis* as a fusion protein, attached to the Cas9 construct) then polymerizes a single strand of DNA which is coded for by the edit template (B) and homology arm (C). The polymerization continues towards the 5′ end of the extension arm. The polymerized strand of ssDNA forms a ssDNA 3′ end flap which, as describe elsewhere (e.g., as shown in FIG. 1G), invades the endogenous DNA, displacing the corresponding endogenous strand (which is removed as a 5′ DNA flap of endogenous DNA), and installing the desired nucleotide edit (single nucleotide base pair change, deletions, insertions (including whole genes) through naturally occurring DNA repair/replication rounds.

[0691] This application of prime editing can be further described in Example 1.

B. Mutagenesis using prime editing with error-prone RT

[0692] In various embodiments, the prime editing system (i.e., prime editing system) may include the use of an error-prone reverse transcriptase for performing targeted mutagenesis, i.e., to mutate only a well-defined stretch of DNA in a genome or other DNA element in a cell. FIG. 22 provides a schematic of an exemplary process for introducing conducting targeted mutagenesis with an error-prone reverse transcriptase on a target locus using a nucleic acid programmable DNA binding protein (napDNAbp) complexed with an extended guide RNA. This process may be referred to as an embodiment of prime editing for targeted mutagenesis. The extended guide RNA comprises an extension at the 3′ or 5′ end of the

guide RNA, or at an intramolecular location in the guide RNA. In step (a), the napDNAbp/gRNA complex contacts the DNA molecule and the gRNA guides the napDNAbp to bind to the target locus to be mutagenized. In step (b), a nick in one of the strands of DNA of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand, i.e., the strand that is not hybridized to the guide RNA sequence. In step (c), the 3′ end DNA strand interacts with the extended portion of the guide RNA in order to prime reverse transcription. In certain embodiments, the 3′ ended DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA. In step (d), an error-prone reverse transcriptase is introduced which synthesizes a mutagenized single strand of DNA from the 3′ end of the primed site towards the 3′ end of the guide RNA. Exemplary mutations are indicated with an asterisk "*". This forms a single-strand DNA flap comprising the desired mutagenized region. In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap (comprising the mutagenized region) such that the desired mutagenized region becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap that forms once the 3′ single strand DNA flap invades and hybridizes to the complementary sequence on the other strand. The process can also be driven towards product formation with second strand nicking, as exemplified in FIG. 1F. Following endogenous DNA repair and/or replication processes, the mutagenized region becomes incorporated into both strands of DNA of the DNA locus.

[0693] This application of prime editing can be further described in Example 2.

[0694] Error-prone or mutagenic RT enzymes are known in the art. As used herein, the term "error-prone" reverse transcriptase refers to a reverse transcriptase enzyme that occurs naturally or which has been derived from another reverse transcriptase (e.g., a wild type M-MLV reverse transcriptase) which has an error rate that is less than the error rate of wild type M-MLV reverse transcriptase. The error rate of wild type M-MLV reverse transcriptase is reported to be in the range of one error in 15,000 to 27,000 nucleobase incorporations. See Boutabout et al. (2001) "DNA synthesis fidelity by the reverse transcriptase of the yeast retrotransposon Ty1," *Nucleic Acids Res* 29(11):2217–2222, which is incorporated herein by reference. Thus, for purposes of this application, the term "error prone" refers to those RT that have an error rate that is greater than one error in 15,000 nucleobase incorporation (6.7 x

10^{-5} or higher), e.g., 1 error in 14,000 nucleobases (7.14 x 10^{-5} or higher), 1 error in 13,000 nucleobases or fewer (7.7 x 10^{-5} or higher), 1 error in 12,000 nucleobases or fewer (7.7 x 10^{-5} or higher), 1 error in 11,000 nucleobases or fewer (9.1 x 10^{-5} or higher), 1 error in 10,000 nucleobases or fewer (1 x 10^{-4} or 0.0001 or higher), 1 error in 9,000 nucleobases or fewer (0.00011 or higher), 1 error in 8,000 nucleobases or fewer (0.00013 or higher) 1 error in 7,000 nucleobases or fewer (0.00014 or higher), 1 error in 6,000 nucleobases or fewer (0.00016 or higher), 1 error in 5,000 nucleobases or fewer (0.0002 or higher), 1 error in 4,000 nucleobases or fewer (0.00025 or higher), 1 error in 3,000 nucleobases or fewer (0.00033 or higher), 1 error in 2,000 nucleobase or fewer (0.00050 or higher), or 1 error in 1,000 nucleobases or fewer (0.001 or higher), or 1 error in 500 nucleobases or fewer (0.002 or higher), or 1 error in 250 nucleobases or fewer (0.004 or higher).

[0695] A variety of mutagenic RTs could be envisioned for generation of mutagenized sequences using prime editing. Two such examples are the mutagenic reverse transcriptases from *Bordetella* phage (see Handa, S., et al. *Nucl Acids Res* 9711-25 (2018), which is incorporated herein by reference) and *Legionella pneumophila* (see Arambula, D., et al. *Proc Natl Acad Sci USA* 8212-7 (2013), which is incorporated by reference). In the case of the RT from *Bordetella* phage (brt), an accessory protein might need to also be added (bavd) to Cas9 – or delivered *in trans* – as well as additional RNA sequences to the PEgRNA to improve binding of the mutagenic RT to the target site (see Handa, S., et al. *Nucl Acids Res* 9711-25 (2018)). When using mutagenic RTs, the template region of the PEgRNA might be enriched in adenosines or AAY codons to enhance diversity.

[0696] The amino acid sequence of the mutagenic RT from *Bordetella* phage is provided as follows. Like other RTs disclosed herein, the Brt protein may be fused to a napDNAbp as a fusion protein to form a functional PE.

Name	Sequence
brt mutagenic rt	MGKRHRNLIDQITTWENLLDAYRKTSHGKRRTWGYLEFKEYDL ANLLALQAELKAGNYERGPYREFLVYEPKPRLISALEFKDRLVQH ALCNIVAPIFEAGLLPYTYACRPDKGTHAGVCHVQAELRRTRATH FLKSDFSKFFPSIDRAALYAMIDKKIHCAATRRLLRVVLPDEGVGI PIGSLTSQLFANVYGGAVDRLLHDELKQRHWARYMDDIVVLGD DPEELRAVFYRLRDFASERLGLKISHWQVAPVSRGINFLGYRIWP THKLLRKSSVKRAKRKVANFIKHGEDESLQRFLASWSGHAQWA DTHNLFTWMEEQYGIACH (SEQ Id no: 235)

[0697] In the case of Brt from *Bodetella*, the PE fusion may also include an additional accessory protein (Bavd). The accessory protein may be fused to the PE fusion protein or

provided *in trans*. The amino acid sequence of Bavd accessory protein is provided as follows:

Name	Sequence
bavd accessory protein to brt	MEPIEEATKCYDQMLIVERYERVISYLYPIAQSIPRKHGVAREMFL KCLLGQVELFIVAGKSNQVSKLYAADAGLAMLRFWLRFLAGIQK PHAMTPHQVETAQVLIAEVGRILGSWIARVNRKGQAGK (SEQ ID NO: 236)

[0698] In the case of Brt from *Bodetella*, the PEgRNA may comprise an additional nucleotide sequence added a PEgRNA, e.g., to the 5′ or 3′ end. Examplary sequence is as follows, which is originally from the *Bordetella phage* genome:

NAME	SEQUENCE
PEGRNA-ADDITION 1	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 237)

[0699] This PEgRNA addition sequence can be reduced in various ways to shorten the length. For example, the PEgRNA-addition 1 sequence could be reduced to the following exemplary alternative addition sequences:

NAME	SEQUENCE
PEGRNA-ADDITION 2	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUC (SEQ ID NO: 238)
PEGRNA-ADDITION 3	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 239)
PEGRNA-ADDITION 4	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCA (SEQ ID NO: 277)
PEGRNA-ADDITION 5	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAACACGUUACA CGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAGGCGGGGCCG ACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 240)
PEGRNA-ADDITION 6	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAACACGUUACA CGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAGGCGGGGCCG ACUUC (SEQ ID NO: 241)
PEGRNA-ADDITION 7	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 242)

PEGRNA-ADDITION 8	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCA (SEQ ID NO: 243)

[0700] In other embodiments, the PEgRNA addition sequence can be also be mutated. For example, the PEgRNA-addition 1 sequence could be mutated to the following exemplary alternative addition sequence:

NAME	SEQUENCE
PEGRNA-ADDITION 1 MUTATED	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGAUGAGCCGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 244)

[0701] In various embodiments relating to the use of PE for introducing mutations, special PEgRNA considerations may apply. For example, without wishing to be bound by theory, the additional PEgRNA sequences described above might be needed to enable efficient mutagenesis via mutagenic RTs.

[0702] Any mutagenic RT may be used with the prime editors disclosed herein. For example, the error-prone RT described in the following references may be used and are incorporated herein by reference:

[0703] Bebenek et al., "Error-prone polymerization by HIV-1 reverse transcriptase. Contribution of template-primer misalignment, miscoding, and termination probability to mutational hot spots.," *J. Biol Chem*, 1993, 268: 10324-34; and

[0704] Menendez-Arias, "Mutation rates and instrinsic fidelity of retroviral reverse transcriptases," 2009, *Viruses*, 1(3): 1137-1165.

[0705] Various error-prone RTs can include, but are not limited to, the following enzymes disclosed in Table 1 of Menendez-Arias et al. (the entire contents of the reference of which are incorporated by reference), as follows:

ERROR-PRONE RT	REPORTED ERROR-RATE RANGE
HIV-1 RT (GROUP M, SUBTYPE B)	0.6 X 10-4 TO 2.0 X 10-4
HIV-1 RT (GROUP O)	5.5 X 10-5
SIV AGM RT	2.9 X 10-5
SIV MNE RT	1.6 X 10-5 TO 1.2 X 10-4
PFV RT	1.7 X 10-4
FIV RT	6.2 X 10-5
AMV RT	5.9 X 10-5
MO-MLV RT	2.7 X 10-5 TO 3.3 X 10-5

C. Use of prime editing for treating triplet expansion disorders

[0706] The prime editing system or prime editing (PE) system described herein may be used to contract trinucleotide repeat mutations (or "triplet expansion diseases") to treating

conditions such as Huntington's disease and other trinucleotide repeat disorders. Trinucleotide repeat expansion disorders are complex, progressive disorders that involve developmental neurobiology and often affect cognition as well as sensori-motor functions. The disorders show genetic anticipation (i.e. increased severity with each generation). The DNA expansions or contractions usually happen meiotically (i.e. during the time of gametogenesis, or early in embryonic development), and often have sex-bias meaning that some genes expand only when inherited through the female, others only through the male. In humans, trinucleotide repeat expansion disorders can cause gene silencing at either the transcriptional or translational level, which essentially knocks out gene function. Alternatively, trinucleotide repeat expansion disorders can cause altered proteins generated with large repetitive amino acid sequences that either abrogate or change protein function, often in a dominant-negative manner (e.g. poly-glutamine diseases).

[0707] Without wishing to be bound by theory, triplet expansion is caused by slippage during DNA replication or during DNA repair synthesis. Because the tandem repeats have identical sequence to one another, base pairing between two DNA strands can take place at multiple points along the sequence. This may lead to the formation of "loop out" structures during DNA replication or DNA repair synthesis. This may lead to repeated copying of the repeated sequence, expanding the number of repeats. Additional mechanisms involving hybrid RNA:DNA intermediates have been proposed. Prime editing may be used to reduce or eliminate these triplet expansion regions by deletion one or more or the offending repeat codon triplets. In an embodiment of this use, FIG. 23, provides a schematic of a PEgRNA design for contracting or reducing trinucleotide repeat sequences with prime editing.

[0708] Prime editing may be implemented to contract triplet expansion regions by nicking a region upstream of the triplet repeat region with the prime editor comprising a PEgRNA appropriated targeted to the cut site. The prime editor then synthesizes a new DNA strand (ssDNA flap) based on the PEgRNA as a template (i.e., the edit template thereof) that codes for a healthy number of triplet repeats (which depends on the particular gene and disease). The newly synthesized ssDNA strand comprising the healthy triplet repeat sequence also is synthesized to include a short stretch of homology (i.e., the homology arm) that matches the sequence adjacent to the other end of the repeat (red strand). Invasion of the newly synthesized strand, and subsequent replacement of the endogenous DNA with the newly synthesized ssDNA flap, leads to a contracted repeat allele.

[0709] Depending on the particular trinucleotide expansion disorder, the defect-inducing triplet expansions may occur in "trinucleotide repeat expansion proteins." Trinucleotide repeat expansion proteins are a diverse set of proteins associated with susceptibility for developing a trinucleotide repeat expansion disorder, the presence of a trinucleotide repeat expansion disorder, the severity of a trinucleotide repeat expansion disorder or any combination thereof. Trinucleotide repeat expansion disorders are divided into two categories determined by the type of repeat. The most common repeat is the triplet CAG, which, when present in the coding region of a gene, codes for the amino acid glutamine (Q). Therefore, these disorders are referred to as the polyglutamine (polyQ) disorders and comprise the following diseases: Huntington Disease (HD); Spinobulbar Muscular Atrophy (SBMA); Spinocerebellar Ataxias (SCA types 1, 2, 3, 6, 7, and 17); and Dentatorubro-Pallidoluysian Atrophy (DRPLA). The remaining trinucleotide repeat expansion disorders either do not involve the CAG triplet or the CAG triplet is not in the coding region of the gene and are, therefore, referred to as the non-polyglutamine disorders. The non-polyglutamine disorders comprise Fragile X Syndrome (FRAXA); Fragile XE Mental Retardation (FRAXE); Friedreich Ataxia (FRDA); Myotonic Dystrophy (DM); and Spinocerebellar Ataxias (SCA types 8, and 12).

[0710] The proteins associated with trinucleotide repeat expansion disorders can be selected based on an experimental association of the protein associated with a trinucleotide repeat expansion disorder to a trinucleotide repeat expansion disorder. For example, the production rate or circulating concentration of a protein associated with a trinucleotide repeat expansion disorder may be elevated or depressed in a population having a trinucleotide repeat expansion disorder relative to a population lacking the trinucleotide repeat expansion disorder. Differences in protein levels may be assessed using proteomic techniques including but not limited to Western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA), and mass spectrometry. Alternatively, the proteins associated with trinucleotide repeat expansion disorders may be identified by obtaining gene expression profiles of the genes encoding the proteins using genomic techniques including but not limited to DNA microarray analysis, serial analysis of gene expression (SAGE), and quantitative real-time polymerase chain reaction (Q-PCR).

[0711] Non-limiting examples of proteins associated with trinucleotide repeat expansion disorders which can be corrected by prime editing include AR (androgen receptor), FMR1 (fragile X mental retardation 1), HTT (huntingtin), DMPK (dystrophia myotonica-protein

kinase), FXN (frataxin), ATXN2 (ataxin 2), ATN1 (atrophin 1), FEN1 (flap structure-specific endonuclease 1), TNRC6A (trinucleotide repeat containing 6A), PABPN1 (poly(A) binding protein, nuclear 1), JPH3 (junctophilin 3), MED15 (mediator complex subunit 15), ATXN1 (ataxin 1), ATXN3 (ataxin 3), TBP (TATA box binding protein), CACNA1A (calcium channel, voltage-dependent, P/Q type, alpha 1A subunit), ATXN80S (ATXN8 opposite strand (non-protein coding)), PPP2R2B (protein phosphatase 2, regulatory subunit B, beta), ATXN7 (ataxin 7), TNRC6B (trinucleotide repeat containing 6B), TNRC6C (trinucleotide repeat containing 6C), CELF3 (CUGBP, Elav-like family member 3), MAB21L1 (mab-21-like 1 (C. elegans)), MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)), TMEM185A (transmembrane protein 185A), SIX5 (SIX homeobox 5), CNPY3 (canopy 3 homolog (zebrafish)), FRAXE (fragile site, folic acid type, rare, fra(X)(q28) E), GNB2 (guanine nucleotide binding protein (G protein), beta polypeptide 2), RPL14 (ribosomal protein L14), ATXN8 (ataxin 8), INSR (insulin receptor), TTR (transthyretin), EP400 (E1A binding protein p400), GIGYF2 (GRB10 interacting GYF protein 2), OGG1 (8-oxoguanine DNA glycosylase), STC1 (stanniocalcin 1), CNDP1 (carnosine dipeptidase 1 (metallopeptidase M20 family)), C10orf2 (chromosome 10 open reading frame 2), MAML3 mastermind-like 3 (Drosophila), DKC1 (dyskeratosis congenita 1, dyskerin), PAXIP1 (PAX interacting (with transcription-activation domain) protein 1), CASK (calcium/calmodulin-dependent serine protein kinase (MAGUK family)), MAPT (microtubule-associated protein tau), SP1 (Sp1 transcription factor), POLG (polymerase (DNA directed), gamma), AFF2 (AF4/FMR2 family, member 2), THBS1 (thrombospondin 1), TP53 (tumor protein p53), ESR1 (estrogen receptor 1), CGGBP1 (CGG triplet repeat binding protein 1), ABT1 (activator of basal transcription 1), KLK3 (kallikrein-related peptidase 3), PRNP (prion protein), JUN (jun oncogene), KCNN3 (potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3), BAX (BCL2-associated X protein), FRAXA (fragile site, folic acid type, rare, fra(X)(q27.3) A (macroorchidism, mental retardation)), KBTBD10 (kelch repeat and BTB (POZ) domain containing 10), MBNL1 (muscleblind-like (Drosophila)), RAD51 (RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)), NCOA3 (nuclear receptor coactivator 3), ERDA1 (expanded repeat domain, CAG/CTG 1), TSC1 (tuberous sclerosis 1), COMP (cartilage oligomeric matrix protein), GCLC (glutamate-cysteine ligase, catalytic subunit), RRAD (Ras-related associated with diabetes), MSH3 (mutS homolog 3 (E. coli)), DRD2 (dopamine receptor D2), CD44 (CD44 molecule (Indian blood group)), CTCF (CCCTC-binding factor (zinc finger protein)), CCND1 (cyclin D1),

CLSPN (claspin homolog (Xenopus laevis)), MEF2A (myocyte enhancer factor 2A), PTPRU (protein tyrosine phosphatase, receptor type, U), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), TRIM22 (tripartite motif-containing 22), WT1 (Wilms tumor 1), AHR (aryl hydrocarbon receptor), GPX1 (glutathione peroxidase 1), TPMT (thiopurine S-methyltransferase), NDP (Norrie disease (pseudoglioma)), ARX (aristaless related homeobox), MUS81 (MUS81 endonuclease homolog (S. cerevisiae)), TYR (tyrosinase (oculocutaneous albinism IA)), EGR1 (early growth response 1), UNG (uracil-DNA glycosylase), NUMBL (numb homolog (Drosophila)-like), FABP2 (fatty acid binding protein 2, intestinal), EN2 (engrailed homeobox 2), CRYGC (crystallin, gamma C), SRP14 (signal recognition particle 14 kDa (homologous Alu RNA binding protein)), CRYGB (crystallin, gamma B), PDCD1 (programmed cell death 1), HOXA1 (homeobox A1), ATXN2L (ataxin 2-like), PMS2 (PMS2 postmeiotic segregation increased 2 (S. cerevisiae)), GLA (galactosidase, alpha), CBL (Cas-Br-M (murine) ecotropic retroviral transforming sequence), FTH1 (ferritin, heavy polypeptide 1), IL12RB2 (interleukin 12 receptor, beta 2), OTX2 (orthodenticle homeobox 2), HOXA5 (homeobox A5), POLG2 (polymerase (DNA directed), gamma 2, accessory subunit), DLX2 (distal-less homeobox 2), SIRPA (signal-regulatory protein alpha), OTX1 (orthodenticle homeobox 1), AHRR (aryl-hydrocarbon receptor repressor), MANF (mesencephalic astrocyte-derived neurotrophic factor), TMEM158 (transmembrane protein 158 (gene/pseudogene)), and ENSG00000078687.

[0712] The prime editors herein disclosed may be used to contract triplet repeat expansion regions in any of the above-indicated disease proteins, including following polyglutamine triplet expansion disease genes (which show the particular location of the pathogenic repeats that may be removed wholly or in part by prime editing):

TRIPLET EXPANSION DISEASE	AFFECTED GENE	POSITION OF PATHOGENIC REPEATS
DENTATORUBROPALLIDOLUYSIAN ATROPHY	ATN1 ATROPHIN-1	49-88
HUNTINGTON'S DISEASE	HTT THE HUNTINGTIN GENE	36-250
SPINAL AND BULBAR MUSCULAR ATROPHY	AR ANDROGEN RECEPTOR	38-62
SPINOCEREBELLAR ATAXIA TYPE 1	ATXN1 ATAXIN 1	49-88

SPINOCEREBELLAR ATAXIA TYPE 2	ATXN2 ATAXIN 2	33-77
SPINOCEREBELLAR ATAXIA TYPE 3	ATXN3 ATAXIN 3	55-86
SPINOCEREBELLAR ATAXIA TYPE 6	CACNA1A	21-30
SPINOCEREBELLAR ATAXIA TYPE 7	ATXN7 ATAXIN 7	38-120
SPINOCEREBELLAR ATAXIA TYPE 17	TBP TATA-BINDING PROTEIN	47-63

[0713] The prime editors herein disclosed may also be used to contract triplet repeat expansion regions typically found in the following non-polyglutamine triplet expansion disease genes:

TRIPLET EXPANSION DISEASE	AFFECTED GENE	POSITION OF PATHOGENIC REPEATS
FRAXA (FRAGILE X SYNDROME)	FMR1 FRAGILE X MENTAL RETARDATION PROTEIN	230+
FXTAS (FRAGILE X-ASSOCIATED TREMOR/ATAXIA SYNDROME)	FMR1	55-200
FRAXE (FRAGILE XE MENTAL RETARDATION)	AFF2	200+
FRDA (FRIEDREICH'S ATAXIA)	FXN FRATAXIN	100+
DM1 (MYOTONIC DYSTROPHY TYPE 1)	DMPK MYOTONIN-PROTEIN KINASE	50+
SCA8 (SPINOCEREBELLAR ATAXIA TYPE 8)	SCA8 ATAXIN 8	110-250
SCA12 (SPINOCEREBELLAR ATAXIA TYPE 12)	PPP2R2B SERINE/THREONIN PROTEIN PHOSPHATASE 2A	66-78

[0714] Prime editing may be implemented to contract triplet expansion regions using a PEgRNA with an edit template that is designed to delete at least one codon of a triplet expansion region. In other embodiments, the PEgRNAs for using in prime editing for this used to delete at least 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20, or 21, or 22, or 23, or 24, or 25, or 26, or 27, or 28, or 29, or 30, or 31, or 32, or 33, or 34, or 35, or 36, or 37, or 38, or 39, or 40, or 41, or 42, or 43, or 44, or 45, or 46, or 47, or 48, or 49, or 50, or 51, or 52, or 53, or 54, or 55, or 56, or

57, or 58, or 59, or 60, or 61, or 62, or 63, or 64, or 65, or 66, or 67, or 68, or 69, or 70, or 71, or 72, or 73, or 74, or 75, or 76, or 77, or 78, or 79, or 80, or 81, or 82, or 83, or 84, or 85, or 86, or 87, or 88, or 89, or 90, or 91, or 92, or 93, or 94, or 95, or 96, or 97, or 98, or 99, or 100, or more codons from a triplet expansion region in order to arrive at a healthy (i.e., not associated with producing the disease) number of triplet repeats.

[0715] In other embodiments, the PEgRNAs for using in prime editing for this used to delete at least 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or 16, or 17, or 18, or 19, or 20 or more codons from a triplet expansion region in order to arrive at a healthy (i.e., not associated with producing the disease) number of triplet repeats.

[0716] In other embodiments, the the PEgRNAs for using in prime editing for this used to delete at least 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or 11, or 12, or 13, or 14, or 15, or more codons from a triplet expansion region in order to arrive at a healthy (i.e., not associated with producing the disease) number of triplet repeats.

[0717] In other embodiments, the PEgRNAs for using in prime editing for this used to delete at least 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or more codons from a triplet expansion region in order to arrive at a healthy (i.e., not associated with producing the disease) number of triplet repeats.

[0718] Prime editing may be configured to correct any triplet expansion region, such as those described in Budworth et al., "A Brief History of Triplet Repeat Diseases," *Methods Mol Biol*, 2013, 1010: 3-17, US 20011/00165540 A1 (Genome editing of genes associated with trinucleotide repeat expansion disorders in animals), US 2016/0355796 A1 (Compositions and methods of use of crispr-cas systems in nucleotide repeat disorders

[0719] In various embodiments, the disclosure provides a prime editing construct suitable for use in a cell having a trinucleotide repeat expansion region in a defective gene comprising (a) a prime editor fusion comprising a napDNAbp and a reverse transcriptase, (b) a PEgRNA comprising a spacer sequence that targets the trinucleotide repeat expansion region and an extension arm comprising an edit template that codes for the removal of the trinucleotide repeat expansion region.

[0720] In various other embodiments, the disclosure provides a method for deleting all or a portion of a trinucleotide repeat expansion region in a defective gene in a cell using prime editing comprising contacting the cell with a prime editor fusion comprising a napDNAbp and a reverse transcriptase and a PEgRNA comprising a spacer sequence that targets the

trinucleotide repeat expansion region and an extension arm comprising an edit template that codes for the removal of the trinucleotide repeat expansion region.

[0721] In various embodiments, the trinucleotide repeat comprises repeating CTG, CAG, CGG, CCG, GAA, or TTC trinucleotides.

[0722] In various other embodiments, the tetranucleotide repeats, pentanucleotide repeats, or hexanucleotide repeats.

D. Use of prime editing for peptide tagging

[0723] In another aspect, the disclosure provides a method of using the herein described prime editors for genetically grafting one or more peptide tags onto a protein using prime editing, More in particular, the disclosure provides a method for genetically installing one or more peptide tags onto a protein comprising: contacting a target nucleotide sequence encoding the protein with a prime editor configured to insert therein a second nucleotide sequence encoding the one or more peptide tags to result in a recombinant nucleotide sequence that encodes a fusion protein comprising the protein fused to the protein tag.

[0724] In other embodiments, the disclosure provides a method for making a fusion protein comprising a peptide of interest and one or more peptide tags, the method comprising: contacting a target nucleotide sequence encoding the protein with a prime editor configured to insert therein a second nucleotide sequence encoding the one or more peptide tags to result in a recombinant nucleotide sequence that encodes the fusion protein comprising the protein fused to the protein tag.

[0725] In various embodiments, the target nucleotide sequence is a specific gene of interest in a genomic DNA. The gene of interest may encode a protein of interest (e.g., a receptor, an enzyme, a therapeutic protein, a membrane protein, a transport protein, a signal transduction protein, or an immunological protein, etc.). The gene of interest may also encode an RNA molecule, including, but not limited to, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), small nuclear RNA (snRNA), antisense RNA, guide RNA, microRNA (miRNA), small interfering RNA (siRNA), and cell-free RNA (cfRNA).

[0726] The peptide tag may be any peptide tag or variant thereof which imparts one or more functions onto a protein for purposes such as separation, purification, visualization, solubilization, or detection. The peptides tags can include “affinity tags” (to facilitate protein purification), “solubilization tags” (to assist in proper folding of proteins), “chromatography tags” (to alter chromatographic properties of proteins), “epitope tags” (to bind to high affinity

antibodies), and "fluorescence tags" (to facilitate visualization of proteins in a cell or in vitro). Examples of peptide tags include, but are not limited to the following tags:

NAME	AMINO ACID SEQUENCE	SEQ ID NO:
AVITAG™	GLNDIFEAQKIEWHE	SEQ ID NO: 245
C-TAG	EPEA	SEQ ID NO: 246
CALMODULIN-TAG	KRRWKKNFIAVSAANRFKKISSSGAL	SEQ ID NO: 247
POLYGLUTAMATE TAG	EEEEEE	SEQ ID NO: 248
E-TAG	GAPVPYPDPLEPR	SEQ ID NO: 249
FLAG-TAG	DYKDDDDK	SEQ ID NO: 2
HA-TAG	YPYDVPDYA	SEQ ID NO: 5
HIS-TAG	H (HIS_1) HH (HIS_2) HHH (HIS_3) HHHH (HIS_4) HHHHH (HIS_5) HHHHHH (HIS_6) HHHHHHH (HIS_7) HHHHHHHH (HIS_8) HHHHHHHHH (HIS_9) HHHHHHHHHH (HIS_{10}) HHHHHHHHHH…H…(HIS_N, WHEREIN N = 1-25)	SEQ ID NO: 252 SEQ ID NO: 253 SEQ ID NO: 254 SEQ ID NO: 255 SEQ ID NO: 256 SEQ ID NO: 257 SEQ ID NO: 258 SEQ ID NO: 259 SEQ ID NO: 260 SEQ ID NO: 261 SEQ ID NO: 262
MYC-TAG	EQKLISEEDL	SEQ ID NO: 6
NE-TAG	TKENPRSNQEESYDDNES	SEQ ID NO: 264
RHO1D4-TAG	TETSQVAPA	SEQ ID NO: 265
S-TAG	KETAAAKFERQHMDS	SEQ ID NO: 266
SBP-TAG	MDEKTTGWRGGHVVEGLAGELEQLRARLEHHPQGQREP	SEQ ID NO: 267
SOFTAG-1	SLAELLNAGLGGS	SEQ ID NO: 268
SOFTAG-2	TQDPSRVG	SEQ ID NO: 269
SPOT-TAG	PDRVRAVSHWSS	SEQ ID NO: 270
STREP-TAG	WSHPQFEK	SEQ ID NO: 271
TC TAG	CCPGCC	SEQ ID NO: 272
TY TAG	EVHTNQDPLD	SEQ ID NO: 273
V5 TAG	GKPIPNPLLGLDST	SEQ ID NO: 3
VSV-TAG	YTDIEMNRLGK	SEQ ID NO: 275
XPRESS TAG	DLYDDDDK	SEQ ID NO: 276

[0727] Peptide tags may also be the following affinity tags (for separation and/or purification of proteins) (as described in Table 9.9.1 of Kimple et al., "Overview of Affinity Tags for Protein Purification," *Curr Protoc Protein Sci*, 2013, 73:Unit-9.9, which is incorporated herein by reference).

NAME	AMINO ACID SEQUENCE	
AU1 EPITOPE	DTYRYI	SEQ ID NO: 278
AU5 EPITOPE	TDFYLK	SEQ ID NO: 279

BACTERIOPHAGE T7 EPITOPE (T7-TAG)	MASMTGGQQMG	SEQ ID NO: 280
BLUETONGUE VIRUS TAG (B-TAG)	QYPALT	SEQ ID NO: 281
E2 EPITOPE	SSTSSDFRDR	SEQ ID NO: 282
HISTIDINE AFFINITY TAG (HAT)	KDHLIHNVHKEFHAHAHNK	SEQ ID NO: 283
HSV EPITOPE	QPELAPED	SEQ ID NO: 284
POLYARGININE (ARG-TAG)	RRRRR	SEQ ID NO: 285
POLYASPARTATE (ASP-TAG)	CCCC	SEQ ID NO: 286
POLYPHENYLALANINE (PHE-TAG)	FFFFFFFFFFF	SEQ ID NO: 287
S1-TAG	NANNPDWDF	SEQ ID NO: 288
S-TAG	KETAAAKFERQHMDS	SEQ ID NO: 266
VSV-G	YTDIEMNRLGK	SEQ ID NO: 275

[0728] In particular embodiments, the peptide tags may include a His^6 tag, FLAG-tag, V5-tag, GCN4-tag, HA-tag, Myc-Tag, FIAsH/ReAsH-tag, Sortase substrate, pi-clamp.

[0729] In various embodiments, the peptide tags may be used for applications that include protein fluorescent labeling, immunoprecipitation, immunoblotting, immunohistochemistry, protein recruitment, inducible protein degrons, and genome-wide screening.

[0730] In various other embodiments, the peptide tag may include an intein sequence to install protein self-splicing function. As used herein, the term "intein" refers to auto-processing polypeptide domains found in organisms from all domains of life. An intein (*int*ervening prot*ein*) carries out a unique auto-processing event known as protein splicing in which it excises itself out from a larger precursor polypeptide through the cleavage of two peptide bonds and, in the process, ligates the flanking extein (external protein) sequences through the formation of a new peptide bond. This rearrangement occurs post-translationally (or possibly co-translationally), as intein genes are found embedded in frame within other protein-coding genes. Furthermore, intein-mediated protein splicing is spontaneous; it requires no external factor or energy source, only the folding of the intein domain. This process is also known as *cis*-protein splicing, as opposed to the natural process of *trans*-protein splicing with "split inteins." Inteins are the protein equivalent of the self-splicing RNA introns (see Perler et al., Nucleic Acids Res. 22:1125-1127 (1994)), which catalyze their own excision from a precursor protein with the concomitant fusion of the flanking protein sequences, known as exteins (reviewed in Perler et al., Curr. Opin. Chem. Biol.

1:292-299 (1997); Perler, F. B. Cell 92(1):1-4 (1998); Xu et al., EMBO J. 15(19):5146-5153 (1996)).

[0731] The mechanism of the protein splicing process has been studied in great detail (Chong, et al., J. Biol. Chem. 1996, 271, 22159-22168; Xu, M-Q & Perler, F. B. EMBO Journal, 1996, 15, 5146-5153) and conserved amino acids have been found at the intein and extein splicing points (Xu, et al., EMBO Journal, 1994, 13 5517-522).

[0732] Inteins can also exist as two fragments encoded by two separately transcribed and translated genes. These so-called split inteins self-associate and catalyze protein- splicing activity in trans. Split inteins have been identified in diverse cyanobacteria and archaea (Caspi et al, Mol Microbiol. 50: 1569-1577 (2003); Choi J. et al, J Mol Biol. 556: 1093-1106 (2006.); Dassa B. et al, Biochemistry. 46:322-330 (2007.); Liu X. and Yang J., J Biol Chem. 275:26315-26318 (2003); Wu H. et al. Proc Natl Acad Sci USA. £5:9226-9231 (1998.); and Zettler J. et al, FEBS Letters. 553:909-914 (2009)), but have not been found in eukaryotes thus far. Recently, a bioinformatic analysis of environmental metagenomic data revealed 26 different loci with a novel genomic arrangement. At each locus, a conserved enzyme coding region is interrupted by a split intein, with a freestanding endonuclease gene inserted between the sections coding for intein subdomains. Among them, five loci were completely assembled: DNA helicases (gp41-l, gp41-8); Inosine-5′-monophosphate dehydrogenase (IMPDH-1); and Ribonucleotide reductase catalytic subunits (NrdA-2 and NrdJ-1). This fractured gene organization appears to be present mainly in phages (Dassa et al, Nucleic Acids Research. 57:2560-2573 (2009)).

[0733] In certain embodiments, the prime editors described herein can be used to insert split-intein tags in two different proteins, causing their intracellular ligation when co-expressed to form a fusion protein. In protein trans-splicing, one precursor protein consists of an N-extein part followed by the N-intein, another precursor protein consists of the C-intein followed by a C-extein part, and a trans-splicing reaction (catalyzed by the N- and C-inteins together) excises the two intein sequences and links the two extein sequences with a peptide bond. Protein trans-splicing, being an enzymatic reaction, can work with very low (e.g., micromolar) concentrations of proteins and can be carried out under physiological conditions.

[0734] The split intein Npu DnaE was characterized as having the highest rate reported for the protein trans-splicing reaction. In addition, the Npu DnaE protein splicing reaction is considered robust and high-yielding with respect to different extein sequences, temperatures from 6 to 37°C, and the presence of up to 6M Urea (Zettler J. et al, FEBS Letters. 553:909-

914 (2009); Iwai I. et al, FEBS Letters 550: 1853-1858 (2006)). As expected, when the Cysl Ala mutation at the N-domain of these inteins was introduced, the initial N to S- acyl shift and therefore protein splicing was blocked. Unfortunately, the C- terminal cleavage reaction was also almost completely inhibited. The dependence of the asparagine cyclization at the C-terminal splice junction on the acyl shift at the N-terminal scissile peptide bond seems to be a unique property common to the naturally split DnaE intein alleles (Zettler J. et al. FEBS Letters. 555:909-914 (2009)).

[0735] Protein trans-splicing, catalyzed by split inteins, provides an entirely enzymatic method for protein ligation. A split-intein is essentially a contiguous intein (e.g. a mini-intein) split into two pieces named N-intein and C-intein, respectively. The N-intein and C-intein of a split intein can associate non-covalently to form an active intein and catalyze the splicing reaction essentially in same way as a contiguous intein does. Split inteins have been found in nature and also engineered in laboratories. As used herein, the term "split intein" refers to any intein in which one or more peptide bond breaks exists between the N-terminal and C-terminal amino acid sequences such that the N-terminal and C-terminal sequences become separate molecules that can non-covalently reassociate, or reconstitute, into an intein that is functional for trans-splicing reactions. Any catalytically active intein, or fragment thereof, may be used to derive a split intein for use in the methods of the invention. For example, in one aspect the split intein may be derived from a eukaryotic intein. In another aspect, the split intein may be derived from a bacterial intein. In another aspect, the split intein may be derived from an archaeal intein. Preferably, the split intein so-derived will possess only the amino acid sequences essential for catalyzing trans-splicing reactions.

[0736] Split inteins may be created from contiguous inteins by engineering one or more split sites in the unstructured loop or intervening amino acid sequence between the -12 conserved beta-strands found in the structure of mini-inteins. Some flexibility in the position of the split site within regions between the beta-strands may exist, provided that creation of the split will not disrupt the structure of the intein, the structured beta-strands in particular, to a sufficient degree that protein splicing activity is lost.

[0737] The prime editors described herein may incorporate peptide tags (including inteins) into the C-terminal end of a protein of interest. In other embodiments, the peptide tags (including inteins) may be incorporated into the N-terminal end of a protein of interest. The peptide tags may also be incorporated into the interior of a protein of interest. The resulting

fusion proteins created by the herein described prime editors may have the following structures:

[0738] [protein of interest] – [peptide tag];

[0739] [peptide tag] – [protein of interest]; or

[0740] [protein of interest – N-terminal region] – [peptide tag] – [protein of interest – C-terminal region].

[0741] The principles of guide RNA design for use in peptide tagging throughout may be applied to peptide tagging. For example, in one embodiment, the PEgRNA structure for peptide tagging may have the following structure: 5′-[spacer sequence]-[gRNA core or scaffold]-[extension arm]-3′, wherein the extension arm comprises in the 5′ to 3′ direction, a homology arm, edit template (comprising the sequence that encodes the peptide tag), and a primer binding site. This configuration is depicted in FIG. 3D and in FIG. 24.

[0742] In another embodiment, the PEgRNA structure for peptide tagging may have the following structure: 5′-[extension arm]-[spacer sequence]-[gRNA core or scaffold]-3′, wherein the extension arm comprises in the 5′ to 3′ direction, a homology arm, edit template (comprising the sequence that encodes the peptide tag), and a primer binding site. This configuration is depicted in FIG. 3E.

[0743] Embodiments of peptide tagging using prime editing is depicted in FIGs. 25 and 26 and described in Example 4.

E. Use of prime editing for preventing or treating prion disease

[0744] Prime editing can also be used to prevent or halt the progression of prion disease through the installation of one or more protective mutations into prion proteins (PRNP) which become misfolded during the course of disease. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce inflammatory response.

[0745] In humans, prion disease includes Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, and Kuru. In animals, prion disease includes Bovine Spongiform Encephalopathy (BSE or “mad cow disease”), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy. Prime editing may be used to install protective point mutations

into a prion protein in order to prevent or halt the progression of any one of these prion diseases.

[0746] Classic CJD is a human prion disease. It is a neurodegenerative disorder with characteristic clinical and diagnostic features. This disease is rapidly progressive and always fatal. Infection with this disease leads to death usually within 1 year of onset of illness. CJD is a rapidly progressive, invariably fatal neurodegenerative disorder believed to be caused by an abnormal isoform of a cellular glycoprotein known as the prion protein. CJD occurs worldwide and the estimated annual incidence in many countries, including the United States, has been reported to be about one case per million population. The vast majority of CJD patients usually die within 1 year of illness onset. CJD is classified as a transmissible spongiform encephalopathy (TSE) along with other prion diseases that occur in humans and animals. In about 85% of patients, CJD occurs as a sporadic disease with no recognizable pattern of transmission. A smaller proportion of patients (5 to 15%) develop CJD because of inherited mutations of the prion protein gene. These inherited forms include Gerstmann-Straussler-Scheinker syndrome and fatal familial insomnia. No treatment is currently known for CJD.

[0747] Variant Creutzfeldt-Jakob disease (vCJD) is a prion disease that was first described in 1996 in the United Kingdom. There is now strong scientific evidence that the agent responsible for the outbreak of prion disease in cows, bovine spongiform encephalopathy (BSE or 'mad cow' disease), is the same agent responsible for the outbreak of vCJD in humans. Variant CJD (vCJD) is not the same disease as classic CJD (often simply called CJD). It has different clinical and pathologic characteristics from classic CJD. Each disease also has a particular genetic profile of the prion protein gene. Both disorders are invariably fatal brain diseases with unusually long incubation periods measured in years, and are caused by an unconventional transmissible agent called a prion. No treatment is currently known for vCJD.

[0748] BSE (bovine spongiform encephalopathy or "mad cow disease") is a progressive neurological disorder of cattle that results from infection by an unusual transmissible agent called a prion. The nature of the transmissible agent is not well understood. Currently, the most accepted theory is that the agent is a modified form of a normal protein known as prion protein. For reasons that are not yet understood, the normal prion protein changes into a pathogenic (harmful) form that then damages the central nervous system of cattle. There is increasing evidence that there are different strains of BSE: the typical or classic BSE strain

responsible for the outbreak in the United Kingdom and two atypical strains (H and L strains). No treatment is currently known for BSE.

[0749] Chronic wasting disease (CWD) is a prion disease that affects deer, elk, reindeer, sika deer and moose. It has been found in some areas of North America, including Canada and the United States, Norway and South Korea. It may take over a year before an infected animal develops symptoms, which can include drastic weight loss (wasting), stumbling, listlessness and other neurologic symptoms. CWD can affect animals of all ages and some infected animals may die without ever developing the disease. CWD is fatal to animals and there are no treatments or vaccines.

[0750] The causative agents of TSEs are believed to be prions. The term “prions” refers to abnormal, pathogenic agents that are transmissible and are able to induce abnormal folding of specific normal cellular proteins called prion proteins that are found most abundantly in the brain. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. Prion diseases are usually rapidly progressive and always fatal.

[0751] As used herein, the term "prion" shall mean an infectious particle known to cause diseases (spongiform encephalopathies) in humans and animals. The term "prion" is a contraction of the words "protein" and "infection" and the particles are comprised largely if not exclusively of $PRNP^{Sc}$ molecules encoded by a PRNP gene which expresses $PRNP^{C}$ which changes conformation to become $PRNP^{Sc}$. Prions are distinct from bacteria, viruses and viroids. Known prions include those which infect animals to cause scrapie, a transmissible, degenerative disease of the nervous system of sheep and goats as well as bovine spongiform encephalopathies (BSE) or mad cow disease and feline spongiform encephalopathies of cats. Four prion diseases, as discussed above, known to affect humans are (1) kuru, (2) Creutzfeldt-Jakob Disease (CJD), (3) Gerstmann-Strassler-Scheinker Disease (GSS), and (4) fatal familial insomnia (FFI). As used herein prion includes all forms of prions causing all or any of these diseases or others in any animals used--and in particular in humans and in domesticated farm animals.

[0752] In general, and without wishing to be bound by theory, prior diseases are caused by misfolding of prion proteins. Such diseases—often called deposition diseases—the misfolding of the prion proteins can be accounted for as follows. If A is the normally synthesized gene product that carries out an intended physiologic role in a monomeric or oligomeric state, A* is a conformationally activated form of A that is competent to undergo a

dramatic conformational change, B is the conformationally altered state that prefers multimeric assemblies (i.e., the misfolded form which forms depositions) and B_n is the multimeric material that is pathogenic and relatively difficult to recycle. For the prion diseases, $PRNP^C$ and $PRNP^{Sc}$ correspond to states A and B_n where A is largely helical and monomeric and B_n is β-rich and multimeric.

[0753] It is known that certain mutations in prion proteins can be associated with increased risk of prior disease. Conversely, certain mutations in prion proteins can be protective in nature. See Bagynszky et al., "Characterization of mutations in PRNP (prion) gene and their possible roles in neurodegenerative diseases," *Neuropsychiatr Dis Treat.*, 2018; 14: 2067-2085, the contents of which are incorporated herein by reference.

[0754] PRNP (NCBI RefSeq No. NP_000302.1 (SEQ ID NO: 291))—the human prion protein--is encoded by a 16 kb long gene, located on chromosome 20 (4686151-4701588). It contains two exons, and the exon 2 carries the open reading frame which encodes the 253 amino acid (AA) long PrP protein. Exon 1 is a noncoding exon, which may serve as transcriptional initiation site. The post-translational modifications result in the removal of the first 22 AA N-terminal fragment (NTF) and the last 23 AA C-terminal fragment (CTF). The NTF is cleaved after PrP transport to the endoplasmic reticulum (ER), while the CTF (glycosylphosphatidylinositol [GPI] signal peptide [GPI-SP]) is cleaved by the GPI anchor. GPI anchor could be involved in PrP protein transport. It may also play a role of attachment of prion protein into the outer surface of cell membrane. Normal PrP is composed of a long N-terminal loop (which contains the octapeptide repeat region), two short β sheets, three α helices, and a C-terminal region (which contains the GPI anchor). Cleavage of PrP results in a 208 AA long glyocoprotein, anchored in the cell membrane.

[0755] The amino acid sequence of PRNP (NP_000302.1) is as follows:

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGG
GWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKPKT
NMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQV
YYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMCITQ
YERESQAYYQRGSSMVLFSSPPVILLISFLIFLIVG (SEQ ID NO: 291).

[0756] The amino acid sequence of PRNP (NP_000302.1) is encoded by the following nucleotide sequence (NCBI Ref. Seq No. NM_000311.5, "homo sapiens prion protein (PRNP), transcript variant 1, mRNA), is as follows:

GCGAACCTTGGCTGCTGGATGCTGGTTCTCTTTGTGGCCACATGGAGTGACCTGG
GCCTCTGCAAGAAGCGCCCGAAGCCTGGAGGATGGAACACTGGGGGCAGCCGAT
ACCCGGGGCAGGGCAGCCCTGGAGGCAACCGCTACCCACCTCAGGGCGGTGGTG
GCTGGGGGCAGCCTCATGGTGGTGGCTGGGGGCAGCCTCATGGTGGTGGCTGGG

GGCAGCCCCATGGTGGTGGCTGGGGACAGCCTCATGGTGGTGGCTGGGGTCAAG
GAGGTGGCACCCACAGTCAGTGGAACAAGCCGAGTAAGCCAAAAACCAACATGA
AGCACATGGCTGGTGCTGCAGCAGCTGGGGCAGTGGTGGGGGGCCTTGGCGGCT
ACATGCTGGGAAGTGCCATGAGCAGGCCCATCATACATTTCGGCAGTGACTATGAG
GACCGTTACTATCGTGAAAACATGCACCGTTACCCCAACCAAGTGTACTACAGGCC
CATGGATGAGTACAGCAACCAGAACAACTTTGTGCACGACTGCGTCAATATCACA
ATCAAGCAGCACACGGTCACCACAACCACCAAGGGGGAGAACTTCACCGAGACC
GACGTTAAGATGATGGAGCGCGTGGTTGAGCAGATGTGTATCACCCAGTACGAGA
GGGAATCTCAGGCCTATTACCAGAGAGGATCGAGCATGGTCCTCTTCTCCTCTCCA
CCTGTGATCCTCCTGATCTCTTTCCTCATCTTCCTGATAGTGGGATGAGGAAGGTCT
TCCTGTTTTCACCATCTTTCTAATCTTTTTCCAGCTTGAGGGAGGCGGTATCCACCT
GCAGCCCTTTTAGTGGTGGTGTCTCACTCTTTCTTCTCTCTTTGTCCCGGATAGGCT
AATCAATACCCTTGGCACTGATGGGCACTGGAAAACATAGAGTAGACCTGAGATG
CTGGTCAAGCCCCCTTTGATTGAGTTCATCATGAGCCGTTGCTAATGCCAGGCCAG
TAAAAGTATAACAGCAAATAACCATTGGTTAATCTGGACTTATTTTTGGACTTAGTG
CAACAGGTTGAGGCTAAAACAAATCTCAGAACAGTCTGAAATACCTTTGCCTGGA
TACCTCTGGCTCCTTCAGCAGCTAGAGCTCAGTATACTAATGCCCTATCTTAGTAGA
GATTTCATAGCTATTTAGAGATATTTTCCATTTTAAGAAAACCCGACAACATTTCTG
CCAGGTTTGTTAGGAGGCCACATGATACTTATTCAAAAAAATCCTAGAGATTCTTA
GCTCTTGGGATGCAGGCTCAGCCCGCTGGAGCATGAGCTCTGTGTGTACCGAGAA
CTGGGGTGATGTTTTACTTTTCACAGTATGGGCTACACAGCAGCTGTTCAACAAGA
GTAAATATTGTCACAACACTGAACCTCTGGCTAGAGGACATATTCACAGTGAACAT
AACTGTAACATATATGAAAGGCTTCTGGGACTTGAAATCAAATGTTTGGGAATGGT
GCCCTTGGAGGCAACCTCCCATTTTAGATGTTTAAAGGACCCTATATGTGGCATTCC
TTTCTTTAAACTATAGGTAATTAAGGCAGCTGAAAAGTAAATTGCCTTCTAGACAC
TGAAGGCAAATCTCCTTTGTCCATTTACCTGGAAACCAGAATGATTTTGACATACA
GGAGAGCTGCAGTTGTGAAAGCACCATCATCATAGAGGATGATGTAATTAAAAAAT
GGTCAGTGTGCAAAGAAAAGAACTGCTTGCATTTCTTTATTTCTGTCTCATAATTG
TCAAAAACCAGAATTAGGTCAAGTTCATAGTTTCTGTAATTGGCTTTTGAATCAAA
GAATAGGGAGACAATCTAAAAAATATCTTAGGTTGGAGATGACAGAAATATGATTG
ATTTGAAGTGGAAAAAGAAATTCTGTTAATGTTAATTAAAGTAAAATTATTCCCTG
AATTGTTTGATATTGTCACCTAGCAGATATGTATTACTTTTCTGCAATGTTATTATTG
GCTTGCACTTTGTGAGTATTCTATGTAAAAATATATATGTATATAAAATATATATTGCA
TAGGACAGACTTAGGAGTTTTGTTTAGAGCAGTTAACATCTGAAGTGTCTAATGCA
TTAACTTTTGTAAGGTACTGAATACTTAATATGTGGGAAACCCTTTTGCGTGGTCCT
TAGGCTTACAATGTGCACTGAATCGTTTCATGTAAGAATCCAAAGTGGACACCATT
AACAGGTCTTTGAAATATGCATGTACTTTATATTTTCTATATTTGTAACTTTGCATGT
TCTTGTTTTGTTATATAAAAAAATTGTAAATGTTTAATATCTGACTGAAATTAAACG
AGCGAAGATGAGCACCA (SEQ ID NO: 292)

[0757] Mutation sites relative to PRNP (NP_000302.1) which are linked to CJD and FFI are reported are as follows. These mutations can be removed or installed using the prime editors disclosed herein.

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO CJD PRION DISEASE (SEE TABLE 1 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)

D178N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHNCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 293)
T188K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHKVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 294)
E196K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGKNFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 295)
E196A	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGANFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 296)
E200K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTKTDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 297)
E200G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTGTDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 298)
V203I	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDIKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPVI LLISFLIFLIVG (SEQ ID NO: 299)
R208H	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMEHVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 300)

V210I	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVIEQMCITQYERESQAYYQRGSSMVLFSSPPVI LLISFLIFLIVG (SEQ ID NO: 301)
E211Q	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVQQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 302)
M232R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSRVLFSSPPVI LLISFLIFLIVG (SEQ ID NO: 303)

[0758] Mutation sites relative to PRNP (NP_000302.1) (SEQ ID NO: 291) which are linked to GSS are reported, as follows:

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO GSS PRION DISEASE (SEE TABLE 2 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)
P102L	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKLSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 304)
P105L	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKLKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 305)
A117V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAVAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 306)
G131V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLVSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 307)

V176G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFGHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 308)
H187R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQRTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 309)
	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 291)
F198S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENSTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 311)
D202N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETNVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 312)
Q212P	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEPMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 313)
Q217R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITRYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 314)
M232T	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSTVLFSSPPVI LLISFLIFLIVG (SEQ ID NO: 315)

[0759] Mutation sites relative to PRNP (NP_000302.1) (SEQ ID NO: 291) which are linked to a possible protective nature against prion disease, as follows:

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO A PROTECTIVE NATURE AGAINST PRION DISEASE (SEE TABLE 4 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)
G127S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGSYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 316)
G127V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGVYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 317)
M129V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYVLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 318)
D167G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMGEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 319)
D167N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMNEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 320)
N171S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSSQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 321)
E219K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYKRESQAYYQRGSSMVLFSSPPV ILLISFLIFLIVG (SEQ ID NO: 322)

P238S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSSPV ILLISFLIFLIVG (SEQ ID NO: 323)

[0760] Thus, in various embodiments, prime editing may be used to remove a mutation in PRNP that is linked to prion disease or install a mutation in PRNP that is considered to be protective against prion disease. For example, prime editing may be use to remove or restore a D178N, V180I, T188K, E196K, E196A, E200K, E200G, V203I, R208H, V210I, E211Q, I215V, or M232R mutation in the PRNP protein (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). In other embodiments, prime editing may be use to remove or restore a P102L, P105L, A117V, G131V, V176G, H187R, F198S, D202N, Q212P, Q217R, or M232T mutation in the PRNP protein (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). By removing or correcting for the presence of such mutations in PRNP using prime editing, the risk of prion disease may be reduced or eliminated.

[0761] In other embodiments, prime editing may be used to install a protective mutation in PRNP that is linked to a protective effect against one or more prion diseases. For example, prime editing may be used to install a G127S, G127V, M129V, D167G, D167N, N171S, E219K, or P238S protective mutation in PRNP (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). In still other embodiments, the protective mutation may be any alternate amino acid installed at G127, G127, M129, D167, D167, N171, E219, or P238 in PRNP (relative to PRNP of NP_000302.1) (SEQ ID NO: 291).

[0762] In particular embodiments, prime editing may be used to install a G127V protective mutation in PRNP, as illustrated in FIG. 27 and discussed in Example 5.

[0763] In another embodiment, prime editing may be used to install an E219K protective mutation in PRNP.

[0764] The *PRNP* protein and the protective mutation site are conserved in mammals, so in addition to treating human disease it could also be used to generate cows and sheep that are immune to prion disease, or even help cure wild populations of animals that are suffering from prion disease. Prime editing has already been used to achieve ~25% installation of a naturally occurring protective allele in human cells, and previous mouse experiments indicate that this level of installation is sufficient to cause immunity from most prion diseases. This method is the first and potentially only current way to install this allele with such high

efficiency in most cell types. Another possible strategy for treatment is to use prime editing to reduce or eliminate the expression of *PRNP* by installing an early stop codon in the gene.

[0765] Using the principles described herein for PEgRNA design, appropriate PEgRNAs may be designed for installing desired protective mutations, or for removing prion disease-associated mutations from PRNP. For example, the below list of PEgRNAs can be used to install the G127V protective allele and the E219K protective allele in human PRNP, as well as the G127V protective allele in PRNP of various animals.

HUMAN PEGRNA FOR INSTALLATION OF G127V IN HUMAN PRNP:	**GCAGTGGTGGGGGGCCTTGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACGCCAA GGCCCCCCAC(SEQ ID NO: 324)
HUMAN PEGRNA FOR INSTALLATION OF E219K IN HUMAN PRNP	**TGTGTATCACCCAGTACGAG**GTTTTAGAGCTAGAAA TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA AAAAGTGGCACCGAGTCGGTGC AGATTCTCTCTT GTACTGGGTGA(SEQ ID NO: 325)
COW (BOS TAURUS) PEGRNA FOR INSTALLATION OF G127V IN COW PRNP	**GCAGTGGTAGGGGGCCTTGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACACCAA GGCCCCCTAC(SEQ ID NO: 326)
HAMSTER (MESOCRICETUS AURATUS) PEGRNA FOR INSTALLATION OF G127V IN HAMSTER PRNP	**GCCGTGGTGGGGGGCCTTGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACACCAA GGCCCCCCAC(SEQ ID NO: 327)
MOUSE (MUS MUSCULUS) PEGRNA FOR INSTALLATION OF G127V IN MOUST PRNP	**GCAGTAGTGGGGGGCCTTGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACACCAA GGCCCCCCAC(SEQ ID NO: 328)
DEER (ODOCOILEUS VIRGINIANUS) PEGRNA FOR INSTALLATION OF G127V IN DEER PNRP	**GCAGTGGTAGGGGGCCTTGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACACCAA GGCCCCCTAC(SEQ ID NO: 329)
FERRET (MUSTELA PUTORIUS FURO) PEGRNA FOR INSTALLATION OF G127V IN FERRET PRNP	**GCGGTTGTGGGGGGCCTGGG**GTTTTAGAGCTAGAA ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGTGCATGTAGACGCCCA GGCCCCCCAC(SEQ ID NO: 330)
KEY:	SPACER IS BOLDED. SGRNA SCAFFOLD IS NORMAL TEXT. RT TEMPLATE IS ONCE UNDERLINED. PBS IS TWICE UNDERLINED.

F. Use of prime editing for RNA tagging

[0766] Prime editing may also be used to manipulate, alter, and otherwise modify the sequences of DNA encoding RNA functions through RNA tagging, and in this way provides a means to indirectly modify the structure and function of RNA. For example, PE can be used to insert motifs that are functional at the RNA level (hereafter RNA motifs) to tag or otherwise manipulate non-coding RNAs or mRNAs. These motifs could serve to increase gene expression, decrease gene expression, alter splicing, change post-transcriptional modification, affect the sub-cellular location of the RNA, enable isolation or determination of the intra- or extra-cellular location of the RNA (using, for instance, fluorescent RNA aptamers such as Spinach, Spinach2, Baby Spinach, or Broccoli), recruit endogenous or exogenous protein or RNA binders, introduce sgRNAs, or induce processing of the RNA, by either self-cleavage or RNAses (see FIG. 28B and Example 6 for further details).

[0767] The following RNA tags or motifs may be inserted into a gene of interest using prime editing with an appropriate PEgRNA (designed using the guidance provided herein) to affect various properties of RNA, including RNA transport, expression level, splicing, and detection.

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
POLYOMAVIRUS SIMIAN VIRUS 40 (SV40) TYPE1	AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTA (SEQ ID NO: 331)	TERMINATION OF TRANSCRIPTION OF THE TAGGED GENE; TRANSPORT OF MRNA INTO CYTOSOL; INCREASED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAGGGCCATGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCC*A* *CCTGAACCGTATATC* **TAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTCTATG*GCCCTGACTGGAA* (SEQ ID NO: 332)

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
POLYOMAVIRUS SIMIAN VIRUS 40 (SV40) TYPE2	CCATGGCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTC (SEQ ID NO: 333)	TERMINATION OF TRANSCRIPTION OF THE TAGGED GENE; TRANSPORT OF MRNA INTO CYTOSOL; INCREASED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAGGGCCATGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCC*A**CCTGAACCGTATATC*** **GAGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTGGGCCATG**GCTATG*GGCCCTGACTGGAA*** (SEQ ID NO: 334)
POLYOMAVIRUS SIMIAN VIRUS 40 (SV40) TYPE3	TGATCATAATCAAGCCATATCACATCTGTAGAGGTTTACTTGCTTTAAAAAACCTCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTGGATCTGC (SEQ ID NO: 335)	TERMINATION OF TRANSCRIPTION OF THE TAGGED GENE; TRANSPORT OF MRNA INTO CYTOSOL; INCREASED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAGGGCCATGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCC*A**CCTGAACCGTATATC*** **GCAGATCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGG**

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			TTCAGGGGGAGG TGTGGAGGTTTTT TAAAGCAAGTAAA CCTCTACAGATGT GATATGGCTTGAT TATGATCACTATG*G CCCTGACTGGAA* (SEQ ID NO: 336)
HUMAN GROWTH HORMONE (HGH)	ACGGGTGGCATCCCTGTG ACCCCTCCCCAGTGCCTC TCCTGGCCCTGGAAGTTG CCACTCCAGTGCCCACCA GCCTTGTCCTAATAAAATT AAGTTGCATCATTTTGTCT GACTAGGTGTCCTTCTATA ATATTATGGGGTGGAGGG GGGTGGTATGGAGCAAGG GGCAAGTTGGGAAGACA ACCTGTAGGGCCTGCGGG GTCTATTGGGAACCAAGC TGGAGTGCAGTGGCACAA TCTTGGCTCACTGCAATCT CCGCCTCCTGGGTTCAAG CGATTCTCCTGCCTCAGCC TCCCGAGTTGTTGGGATT CCAGGCATGCATGACCAG GCTCAGCTAATTTTTGTTT TTTTGGTAGAGACGGGGT TTCACCATATTGGCCAGGC TGGTCTCCAACTCCTAATC TCAGGTGATCTACCCACCT TGGCCTCCCAAATTGCTG GGATTACAGGCGTGAACC ACTGCTCCCTTCCCTGTCC TT (SEQ ID NO: 337)	TRANSPORT OF RNA INTO CYTOPLASM; ENHANCED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **AAGGACAGGGAA GGGAGCAGTGGT TCACGCCTGTAAT CCCAGCAATTTGG GAGGCCAAGGTG GGTAGATCACCTG AGATTAGGAGTTG GAGACCAGCCTG GCCAATATGGTGA AACCCCGTCTCTA CCAAAAAAACAAA AATTAGCTGAGCC TGGTCATGCATGC CTGGAATCCCAAC AACTCGGGAGGCT GAGGCAGGAGAAT CGCTTGAACCCAG GAGGCGGAGATTG CAGTGAGCCAAGA TTGTGCCACTGCA CTCCAGCTTGGTT CCCAATAGACCCC GCAGGCCCTACAG GTTGTCTTCCCAA CTTGCCCCTTGCT CCATACCACCCCC CTCCACCCCATAA**

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			TATTATAGAAGGA CACCTAGTCAGAC AAAATGATGCAAC TTAATTTTATTAGG ACAAGGCTGGTG GGCACTGGAGTG GCAACTTCCAGGG CCAGGAGAGGCA CTGGGGAGGGGT CACAGGGATGCCA CCCGTCTATG*GCCC TGACTGGAA* (SEQ ID NO: 338)
BOVINE GROWTH HORMONE (BGH)	CGACTGTGCCTTCTAGTTG CCAGCCATCTGTTGTTTGC CCCTCCCCCGTGCCTTCCT TGACCCTGGAAGGTGCCA CTCCCACTGTCCTTTCCTA ATAAAATGAGGAAATTGC ATCGCATTGTCTGAGTAGG TGTCATTCTATTCTGGGGG GTGGGGTGGGGCAGGAC AGCAAGGGGGAGGATTG GGAAGACAATAGCAGGCA TGCTGGGGATGCGGTGGG CTCTATGG (SEQ ID NO: 339)	TRANSPORT OF RNA INTO CYTOPLASM; ENHANCED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **CCATAGAGCCCAC CGCATCCCCAGCA TGCCTGCTATTGT CTTCCCAATCCTC CCCCTTGCTGTCC TGCCCCACCCCAC CCCCCAGAATAGA ATGACACCTACTC AGACAATGCGATG CAATTTCCTCATTT TATTAGGAAAGGA CAGTGGGAGTGG CACCTTCCAGGGT CAAGGAAGGCAC GGGGGAGGGGCA AACAACAGATGGC TGGCAACTAGAAG GCACAGTCG**CTAT G*GCCCTGACTGGAA* (SEQ ID NO: 340)
RABBIT BETA-	TTCACTCCTCAGGTGCAG GCTGCCTATCAGAAGGTG	TRANSPORT OF RNA INTO	ATCCTTCCAGTCAG GGCCATGTTTGAGA

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
GLOBIN (RBGLOB)	GTGGCTGGTGTGGCCAAT GCCCTGGCTCACAAATAC CACTGAGATCTTTTTCCCT CTGCCAAAAATTATGGGG ACATCATGAAGCCCCTTG AGCATCTGACTTCTGGCTA ATAAAGGAAATTTATTTTC ATTGCAATAGTGTGTTGGA ATTTTTTGTGTCTCTCACT CGGAAGGACATATGGGAG GGCAAATCATTTAAAACAT CAGAATGAGTATTTGGTTT AGAGTTTGGCAACATATG CCCATATGCTGGCTGCCAT GAACAAAGGTTGGCTATA AAGAGGTCATCAGTATATG AAACAGCCCCCTGCTGTC CATTCCTTATTCCATAGAA AAGCCTTGACTTGAGGTT AGATTTTTTTTATATTTTGT TTTGTGTTATTTTTTTCTTT AACATCCCTAAAATTTTCC TTACATGTTTTACTAGCCA GATTTTTCCTCCTCTCCTG ACTACTCCCAGTCATAGCT GTCCCTCTTCTCTTATGGA GATC (SEQ ID NO: 341)	CYTOPLASM; ENHANCED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCCA *CCTGAACCGTATATC* **GATCTCCATAAGA GAAGAGGGACAG CTATGACTGGGAG TAGTCAGGAGAGG AGGAAAAATCTGG CTAGTAAAACATG TAAGGAAAATTTT AGGGATGTTAAAG AAAAAAATAACAC AAAACAAAATATA AAAAAAATCTAAC CTCAAGTCAAGGC TTTTCTATGGAATA AGGAATGGACAGC AGGGGGCTGTTTC ATATACTGATGAC CTCTTTATAGCCA ACCTTTGTTCATG GCAGCCAGCATAT GGGCATATGTTGC CAAACTCTAAACC AAATACTCATTCT GATGTTTTAAATG ATTTGCCCTCCCA TATGTCCTTCCGA GTGAGAGACACAA AAAATTCCAACAC ACTATTGCAATGA AAATAAATTTCCTT TATTAGCCAGAAG TCAGATGCTCAAG GGGCTTCATGATG TCCCCATAATTTTT GGCAGAGGGAAA AAGATCTCAGTGG TATTTGTGAGCCA GGGCATTGGCCAC**

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			ACCAGCCACCACC TTCTGATAGGCAG CCTGCACCTGAGG AGTGAACTATG*GCC CTGACTGGAA* (SEQ ID NO: 342)
THYMIDINE KINASE (TK)	GGGGGAGGCTAACTGAAA CACGGAAGGAGACAATAC CGGAAGGAACCCGCGCTA TGACGGCAATAAAAAGAC AGAATAAAACGCACGGGT GTTGGGTCGTTTGTTCATA AACGCGGGGTTCGGTCCC AGGGCTGGCACTCTGTCG ATACCCCACCGAGACCCC ATTGGGGCCAATACGCCC GCGTTTCTTCCTTTTCCCC ACCCCACCCCCCAAGTTC GGGTGAAGGCCCAGGGCT CGCAGCCAACGTCGGGGC GGCAGGCCCTGCCATAG (SEQ ID NO: 343)	TRANSPORT OF RNA INTO CYTOPLASM; ENHANCED RNA STABILITY AND EXPRESSION OF ENCODED PROTEIN	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **CTATGGCAGGGCC TGCCGCCCCGACG TTGGCTGCGAGCC CTGGGCCTTCACC CGAACTTGGGGG GTGGGGTGGGGA AAAGGAAGAAAC GCGGGCGTATTGG CCCCAATGGGGTC TCGGTGGGGTATC GACAGAGTGCCA GCCCTGGGACCG AACCCCGCGTTTA TGAACAAACGACC CAACACCCGTGCG TTTTATTCTGTCTT TTTATTGCCGTCA TAGCGCGGGTTCC TTCCGGTATTGTC TCCTTCCGTGTTT CAGTTAGCCTCCC CC**CTATG*GCCCTGA CTGGAA* (SEQ ID NO: 344)
MALAT1 ENE-MASCRNA	TAGGGTCATGAAGGTTTTT CTTTTCCTGAGAAAACAA CACGTATTGTTTTCTCAGG TTTTGCTTTTTGGCCTTTT TCTAGCTTAAAAAAAAAA	RESULTS IN RETENTION OF RNA IN NUCLEUS, TRANSCRIPT	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
	AAAGCAAAAGATGCTGGT GGTTGGCACTCCTGGTTT CCAGGACGGGGTTCAAAT CCCTGCGGCGTCTTTGCTT TGACT (SEQ ID NO: 345)	TERMINATIO N AND STABILIZATI ON	TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **AGTCAAAGCAAAG ACGCCGCAGGGAT TTGAACCCCGTCC TGGAAACCAGGA GTGCCAACCACCA GCATCTTTTGCTT TTTTTTTTTTTAAG CTAGAAAAAGGCC AAAAAGCAAAACC TGAGAAAACAATA CGTGTTGTTTTCT CAGGAAAAGAAAA ACCTTCATGACCC TA**CTATG*GCCCTGA CTGGAA* (SEQ ID NO: 346)
KSHV PAN ENE	TGTTTTGGCTGGGTTTTTC CTTGTTCGCACCGGACAC CTCCAGTGACCAGACGGC AAGGTTTTTATCCCAGTGT ATATTGGAAAAACATGTTA TACTTTTGACAATTTAACG TGCCTAGAGCTCAAATTA AACTAATACCATAACGTAA TGCAACTTACAACATAAAT AAAGGTCAATGTTTAATCC ATAAAAAAAAAAAAAAA AAAA (SEQ ID NO: 347)	RESULTS IN RETENTION OF RNA IN NUCLEUS, TRANSCRIPT TERMINATIO N AND STABILIZATI ON	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **TTTTTTTTTTTTT TTTTATGGATTAA ACATTGACCTTTA TTTATGTTGTAAG TTGCATTACGTTAT GGTATTAGTTTAAT TTGAGCTCTAGGC ACGTTAAATTGTC AAAAGTATAACAT GTTTTTCCAATATA CACTGGGATAAAA ACCTTGCCGTCTG GTCACTGGAGGTG TCCGGTGCGAACA AGGAAAAACCCAG**

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			CCAAAACACTATG *GCCCTGACTGGAA* (SEQ ID NO: 348)
THREE, SEQUENTIAL KSHV PAN ENES WITH SHORT, UNCONSERVED RNA LINKERS	TGTTTTGGCTGGGTTTTTC CTTGTTCGCACCGGACAC CTCCAGTGACCAGACGGC AAGGTTTTTATCCCAGTGT ATATTGGAAAAACATGTTA TACTTTTGACAATTTAACG TGCCTAGAGCTCAAATTA AACTAATACCATAACGTAA TGCAACTTACAACATAAAT AAAGGTCAATGTTTAATCC ATAAAAAAAAAAAAAAA AAAAACACACTGTTTTGG CTGGGTTTTTCCTTGTTCG CACCGGACACCTCCAGTG ACCAGACGGCAAGGTTTT TATCCCAGTGTATATTGGA AAAACATGTTATACTTTTG ACAATTTAACGTGCCTAG AGCTCAAATTAAACTAATA CCATAACGTAATGCAACTT ACAACATAAATAAAGGTC AATGTTTAATCCATAAAAA AAAAAAAAAAAAAATCTC TCTGTTTTGGCTGGGTTTT TCCTTGTTCGCACCGGAC ACCTCCAGTGACCAGACG GCAAGGTTTTTATCCCAGT GTATATTGGAAAAACATGT TATACTTTTGACAATTTAA CGTGCCTAGAGCTCAAAT TAAACTAATACCATAACGT AATGCAACTTACAACATA AATAAAGGTCAATGTTTA ATCCATAAAAAAAAAAAA AAAAAAA (SEQ ID NO: 349)	RESULTS IN RETENTION OF RNA IN NUCLEUS, TRANSCRIPT TERMINATIO N AND STABILIZATI ON, PREDICTED TO BE GREATER THAN A SINGLE PAN ENE	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A* *CCTGAACCGTATATC* **TTTTTTTTTTTTT TTTTTATGGATTAA ACATTGACCTTTA TTTATGTTGTAAG TTGCATTACGTTAT GGTATTAGTTTAAT TTGAGCTCTAGGC ACGTTAAATTGTC AAAAGTATAACAT GTTTTTCCAATATA CACTGGGATAAAA ACCTTGCCGTCTG GTCACTGGAGGTG TCCGGTGCGAACA AGGAAAAACCCAG CCAAAACAGAGAG ATTTTTTTTTTTT TTTTTATGGATTA AACATTGACCTTT ATTTATGTTGTAAG TTGCATTACGTTAT GGTATTAGTTTAAT TTGAGCTCTAGGC ACGTTAAATTGTC AAAAGTATAACAT GTTTTTCCAATATA CACTGGGATAAAA ACCTTGCCGTCTG GTCACTGGAGGTG TCCGGTGCGAACA AGGAAAAACCCAG CCAAAACAGTGTG**

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			TTTTTTTTTTTTT TTTTTATGGATTA AACATTGACCTTT ATTTATGTTGTAAG TTGCATTACGTTAT GGTATTAGTTTAAT TTGAGCTCTAGGC ACGTTAAATTGTC AAAAGTATAACAT GTTTTTCCAATATA CACTGGGATAAAA ACCTTGCCGTCTG GTCACTGGAGGTG TCCGGTGCGAACA AGGAAAAACCCAG CCAAAACACTATG *GCCCTGACTGGAA* (SEQ ID NO: 350)
SMBOX/U1 SNRNA BOX	CAGCAAGTTCAGAGAAAT CTGAACTTGCTGGATTTTT GGAGCAGGGAGATGGAAT AGGAGCTTGCTCCGTCCA CTCCACGCATCGACCTGG TATTGCAGTACCTCCAGG AACGGTGCACCCACTTTC TGGAGTTTCAAAAGTAGA CTGTACGCTAAGGGTCATA TCTTTTTTTGTTTGGTTTG TGTCTTGGTTGGCGTCTTA AA (SEQ ID NO: 351)	RESULTS IN RETENTION OF RNA IN NUCLEUS AND TRANSCRIPT TERMINATIO N	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A* *CCTGAACCGTATATC* **TTTAAGACGCCAA CCAAGACACAAAC CAAACAAAAAAAG ATATGACCCTTAG CGTACAGTCTACT TTTGAAACTCCAG AAAGTGGGTGCAC CGTTCCTGGAGGT ACTGCAATACCAG GTCGATGCGTGGA GTGGACGGAGCA AGCTCCTATTCCA TCTCCCTGCTCCA AAAATCCAGCAAG TTCAGATTTCTCT GAACTTGCTG**CTA

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			TG*GCCCTGACTGGA A* (SEQ ID NO: 352)
U1 SNRNA 3′ BOX	GTTTCAAAAGTAGACTGT ACGCTAAGGGTCATATCTT TTTTTGTTTGGTTTGTGTC TTGGTTGGCGTCTTAAA (SEQ ID NO: 353)	RESULTS IN RETENTION OF RNA IN NUCLEUS AND TRANSCRIPT TERMINATIO N	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **TTTAAGACGCCAA CCAAGACACAAAC CAAACAAAAAAAG ATATGACCCTTAG CGTACAGTCTACT TTTGAAAC**CTATG*G CCCTGACTGGAA* (SEQ ID NO: 354)
TRNA-LYSINE	GCCCGGCTAGCTCAGTCG GTAGAGCATGAGACTCTT AATCTCAGGGTCGTGGGT TCGAGCCCCACGTTGGGC G (SEQ ID NO: 355)	REPORTED TO ENABLE TRANSPORT OF RNA TO MITOCHOND RIA	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **CGCCCAACGTGG GGCTCGAACCCAC GACCCTGAGATTA AGAGTCTCATGCT CTACCGACTGAGC TAGCCGGGC**CTAT G*GCCCTGACTGGAA* (SEQ ID NO: 356)
BROCCOLI APTAMER	GAGACGGTCGGGTCCAGA TATTCGTATCTGTCGAGTA GAGTGTGGGCTC (SEQ ID NO: 357)	VISUALIZATI ON (FLUORESCE NCE)	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC*

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
			GAGCCCACACTCT ACTCGACAGATAC GAATATCTGGACC CGACCGTCTCCTA TG*GCCCTGACTGGA A* (SEQ ID NO: 358)
SPINACH APTAMER	GACGCAACTGAATGAAAT GGTGAAGGACGGGTCCA GGTGTGGCTGCTTCGGCA GTGCAGCTTGTTGAGTAG AGTGTGAGCTCCGTAACT AGTCGCGTC (SEQ ID NO: 359)	VISUALIZATION (FLUORESCENCE)	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **GACGCGACTAGTT ACGGAGCTCACAC TCTACTCAACAAG CTGCACTGCCGAA GCAGCCACACCTG GACCCGTCCTTCA CCATTTCATTCAG TTGCGTC**CTATG*GC CCTGACTGGAA* (SEQ ID NO: 360)
SPINACH2 APTAMER	GATGTAACTGAATGAAAT GGTGAAGGACGGGTCCA GTAGGCTGCTTCGGCAGC CTACTTGTTGAGTAGAGT GTGAGCTCCGTAACTAGT TACATC (SEQ ID NO: 361)	VISUALIZATION (FLUORESCENCE)	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **GATGTAACTAGTT ACGGAGCTCACAC TCTACTCAACAAG TAGGCTGCCGAAG CAGCCTACTGGAC CCGTCCTTCACCA TTTCATTCAGTTA CATC**CTATG*GCCCT GACTGGAA* (SEQ ID NO: 362)

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
MANGO APTAMER	GGCACGTACGAAGGGACG GTGCGGAGAGGAGAGTAC GTGC (SEQ ID NO: 363)	VISUALIZATION (FLUORESCENCE)	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **GCACGTACTCTCC TCTCCGCACCGTC CCTTCGTACGTGC C**CTATG*GCCCTGAC TGGAA* (SEQ ID NO: 364)
HDV RIBOZYME	GGCCGGCATGGTCCCAGC CTCCTCGCTGGCGCCGGC TGGGCAACATGCTTCGGC ATGGCGAATGGGAC (SEQ ID NO: 365)	3′ END RNA PROCESSING	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **GTCCCATTCGCCA TGCCGAAGCATGT TGCCCAGCCGGC GCCAGCGAGGAG GCTGGGACCATGC CGGCC**CTATG*GCCC TGACTGGAA* (SEQ ID NO: 366)
N^6-METHYLADENOSINE MARKER (M^6A)	GGACTCTAGGACTGGACT TTGGACT (SEQ ID NO: 367)	TARGET FOR METHYLATION (UNDERLINED A'S ARE METHYLATED). M6A METHYLATION CAN RESULT IN ENHANCED RNA STABILITY	ATCCTTCCAGTCAG GGCCATGTTTGAGA GCTAGAAATAGCAA GTTTAAATAAGGCT AGTCCGTTATCAAC TTGAAAAAGTGGG ACCGAGTCGGTCC*A CCTGAACCGTATATC* **AGTCCAAAGTCCA GTCCTAGAGTCC**C TATG*GCCCTGACTG GAA* (SEQ ID NO: 368)

RNA MOTIF	SEQUENCE OF RNA MOTIF	FUNCTION/ EFFECT	EXEMPLARY PEGRNA FOR PRIME EDITING INSERTION OF RNA MOTIF INTO THE EXEMPLARY HEXA GENE*
		AND EXPRESSION, BUT IS NOT YET FULLY UNDERSTOO D	

* each PEgRNA is shown in the 5′ to 3′ direction and has the following structural elements of FIG. 3F as designated by font type, as follows: 5′ – spacer sequence (normal font) – gRNA core (underlined sequence) – homology arm (italicized) – RT template (bolded font) – primer binding site (italicized) – 3′.

[0768] The PEgRNAs of the above table are designed to site-specifically insert examples of the above motifs into the *HEXA* gene (defective in Tay-Sachs disease) (e.g., GenBank No. KR710351.1 (SEQ ID NO: 369), however, this is only for purposes of illustration. The use of prime editing in RNA tagging is not limited to the *HEXA* gene and indeed may be any any. The *HEXA* mRNA has the following nucleotide sequence:

GTTCGTTGCAACAAATTGATGAGCAATGCTTTTTTATAATGCCAACTTTGTACAAA
AAAGTTGGCATGACAAGTTCCAGGCTTTGGTTTTCGCTGCTGCTGGCGGCAGCGT
TCGCAGGACGGGCGACGGCCCTCTGGCCCTGGCCTCAGAACTTCCAAACCTCCG
ACCAGCGCTACGTCCTTTACCCGAACAACTTTCAATTCCAGTACGATGTCAGCTCG
GCCGCGCAGCCCGGCTGCTCAGTCCTCGACGAGGCCTTCCAGCGCTATCGTGACC
TGCTTTTCGGTTCCGGGTCTTGGCCCCGTCCTTACCTCACAGGGAAACGGCATACA
CTGGAGAAGAATGTGTTGGTTGTCTCTGTAGTCACACCTGGATGTAACCAGCTTCC
TACTTTGGAGTCAGTGGAGAATTATACCCTGACCATAAATGATGACCAGTGTTTAC
TCCTCTCTGAGACTGTCTGGGGAGCTCTCCGAGGTCTGGAGACTTTTAGCCAGCT
TGTTTGGAAATCTGCTGAGGGCACATTCTTTATCAACAAGACTGAGATTGAGGACT
TTCCCCGCTTTCCTCACCGGGGCTTGCTGTTGGATACATCTCGCCATTACCTGCCAC
TCTCTAGCATCCTGGACACTCTGGATGTCATGGCGTACAATAAATTGAACGTGTTC
CACTGGCATCTGGTAGATGATCCTTCCTTCCCATATGAGAGCTTCACTTTTCCAGAG
CTCATGAGAAAGGGGTCCTACAACCCTGTCACCCACATCTACACAGCACAGGATG
TGAAGGAGGTCATTGAATACGCACGGCTCCGGGGTATCCGTGTGCTTGCAGAGTT
TGACACTCCTGGCCACACTTTGTCCTGGGGACCAGGTATCCCTGGATTACTGACTC
CTTGCTACCCTGGGTCTGAGCCCTCTGGCACCTTTGGACCAGTGAATCCCAGTCTC

AATAATACCTATGAGTTCATGAGCACATTCTTCTTAGAAGTCAGCTCTGTCTTCCCA
GATTTTTATCTTCATCTTGGAGGAGATGAGGTTGATTTCACCTGCTGGAAGTCCAA
CCCAGAGATCCAGGACTTTATGAGGAAGAAAGGCTTCGGTGAGGACTTCAAGCA
GCTGGAGTCCTTCTACATCCAGACGCTGCTGGACATCGTCTCTTCTTATGGCAAGG
GCTATGTGGTGTGGCAGGAGGTGTTTGATAATAAAGTAAAGATTCAGCCAGACAC
AATCATACAGGTGTGGCGAGAGGATATTCCAGTGAACTATATGAAGGAGCTGGAA
CTGGTCACCAAGGCCGGCTTCCGGGCCCTTCTCTCTGCCCCCTGGTACCTGAACC
GTATATCCTATGGCCCTGACTGGAAGGATTTCTACGTAGTGGAACCCCTGGCATTTG
AAGGTACCCCTGAGCAGAAGGCTCTGGTGATTGGTGGAGAGGCTTGTATGTGGGG
AGAATATGTGGACAACACAAACCTGGTCCCCAGGCTCTGGCCCAGAGCAGGGGC
TGTTGCCGAAAGGCTGTGGAGCAACAAGTTGACATCTGACCTGACATTTGCCTAT
GAACGTTTGTCACACTTCCGCTGTGAGTTGCTGAGGCGAGGTGTCCAGGCCCAAC
CCCTCAATGTAGGCTTCTGTGAGCAGGAG
TTTGAACAGACCTGCCCAACTTTCTTGTACAAAGTTGGCATTATAAGAAAGCATTG
CTTATCAATTTGTTGCAACGAAC (SEQ ID NO: 369).

[0769] The corresponding HEXA protein has the following amino acid sequence:
MTSSRLWFSLLLAAAFAGRATALWPWPQNFQTSDQRYVLYPNNFQFQYDVSSAAQP
GCSVLDEAFQRYRDLLFGSGSWPRPYLTGKRHTLEKNVLVVSVVTPGCNQLPTLESV
ENYTLTINDDQCLLLSETVWGALRGLETFSQLVWKSAEGTFFINKTEIEDFPRFPHRGL
LLDTSRHYLPLSSILDTLDVMAYNKLNVFHWHLVDDPSFPYESFTFPELMRKGSYNP
VTHIYTAQDVKEVIEYARLRGIRVLAEFDTPGHTLSWGPGIPGLLTPCYPGSEPSGTFG
PVNPSLNNTYEFMSTFFLEVSSVFPDFYLHLGGDEVDFTCWKSNPEIQDFMRKKGFG
EDFKQLESFYIQTLLDIVSSYGKGYVVWQEVFDNKVKIQPDTIIQVWREDIPVNYMK
ELELVTKAGFRALLSAPWYLNRISYGPDWKDFYVVEPLAFEGTPEQKALVIGGEACM
WGEYVDNTNLVPRLWPRAGAVAERLWSNKLTSDLTFAYERLSHFRCELLRRGVQAQP
LNVGFCEQEFEQT (SEQ ID NO: 370).

[0770] Notably, the resulting RNA motifs would be included within the translated region of the *HEXA* gene, disrupting the function of the protein coding gene. Inserted polyadenylation motifs would result in premature transcript termination. This site is merely illustratrative of the potential PEgRNAs that could result in insertion of the listed RNA motifs of the above table within a genomic site that is transcribed and thus which would produce an RNA product.

[0771] PEgRNAs for use with PE for RNA tagging could be be expressed from a U6 promoter (in which case a single guanosine would be added to the 5′ end of the PEgRNA for guides that include protospacers that do not begin with a G and 6-7 thymine would be added to the 3′ end) or a pol II promoter such as pCMV (in which case it might be necessary to remove the intrinsically transcribed sequence of this promoter from the 5′ end of the RNA via a self-cleaving element or Csy4 motif, and a termination motif would need to be added to the 3′ end of the RNA that does not result in export of the RNA from the nucleus, such as the 3′ box motif listed above. Note that this motif would *not* be inserted into the genome as a result of PE, as it would be 3′ of the annealing region). The core PEgRNA scaffold is underlined, the homology and annealing regions are italicized, and the inserted sequence is bolded. Note that the sequence inserted is the reverse complement of the above examples – as described below and therefore these PEgRNAs would need to be targeted to the coding strand.

[0772] Also, note that self-cleaving ribozymes other than HDV need in some embodiments to be tailored to the given target site; that is, while HDV cleaves the encoded transcript immediately 5′ to itself, the cut sites for all other self-cleaving ribozymes are within the ribozyme itself. Therefore, the first and last roughly 5-10 nucleotides (and in some instances potentially more than 10) would actually be a part of the encoded sequence. As an example, to cleave the sequence 5′NNNNNTCATCCTGATAAACTGCAAA3′ (SEQ ID NO: 371) after the 5 Ns, where N is any nucleotide, using a hammerhead self-cleaving ribozyme, the following sequence would be inserted, where the underlined sequences form an imperfect RNA pairing element.

[0773] 5′NNNNN<u>CAGTTTGTACGGA</u>TGACTGATGAGTCCCAAATAGGACGAAACGC GCTTCGGTGCGTCTCATCCTGATAAACTGCAAA-3′ (SEQ ID NO: 372).

[0774] There is significant flexibility in terms of the length and nature of this pairing element, and this would be true for any of the non-HDV self-cleaving ribozymes listed in the original submission. To install a hammerhead ribozyme to cleave the hexA mRNA using a PEgRNA with the same protospacer as the above listed constructs, the following PEgRNA sequence could be used (labels same as above):

[0775] 5′ACCTGAACCGTATATCGACGCACCGAAGCGCGTTTCGTCCTATTTGGGAC TCATCAGGATATACGGTTCAGGTGATATACGGTTCAGGTGACGCACCGAAGCGCGT TTCGTCCTATTTGGGACTCATCAGACCTGAACCGTATATCATCCTTCCAGTCAGGGC CAT<u>GTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTG AAAAAGTGGGACCGAGTCGGTCC</u>ACCTGAACCGTATATC**GACGCACCGAAGCGC**

GTTTCGTCCTATTTGGGACTCATCAGGATATACGGTTCAGGTCTATGGCCCTGA CTGGAA-3′ (wherein the core PEgRNA scaffold is underlined, the homology and annealing regions are italicized, and the inserted sequence is bolded) (SEQ ID NO: 373).

[0776] Designing other PEgRNA for insertion of RNA motifs may follow the general principle described herein. However, it is noted that many of RNA motifs are potentially highly structured, which could make it difficult for them to be reverse-transcribed and inserted into the genome. Although for some RNA sequences, such as simple hairpins, both the RNA sequence itself and its complement are structured. However, that is unlikely to be true for the sequences noted above. Therefore, when inserting these motifs, it would most likely be best for the PEgRNA to encode the reverse complement of these sequences, resulting in the insertion of the DNA sequence actually encoding the motif into the genome. Similarly, inclusion of a self-cleaving ribozyme in the PEgRNA template region would result in processing and inefficient activity, while inclusion of its reverse complement would not. Thus, these PEgRNAs will likely have to target the coding strand, whereas PEgRNAs encoding other types of insertions (such as therapeutic correction) would be able to theoretically target either strand.

[0777] Also, note that for many of the inserted motifs, the resulting PEgRNA might not be able to be transcribed from the U6 promoter, necessitating use of other promoters, such as pCMV. Similarly, longer PEgRNAs could also be less stable. Shorter motifs, such as m^6A markers, would not have this challenge.

G. Use of prime editing for generation of sophisticated gene libraries

[0778] Prime editing may also be used to generate sophisticated libraries of protein- or RNA-coding genes with defined or variable insertions, deletions, or defined amino acid/nucleotide conversions, and their use in high-throughput screening and directed evolution is described herein. This application of prime editing can be further described in Example 7.

[0779] The generation of variable genetic libraries has most commonly been accomplished through mutagenic PCR (see Cadwell RC and Joyce GF. PCR Methods Appl. 1992). This method relies on either using reaction conditions that reduce the fidelity of DNA polymerase, or using modified DNA polymerases with higher mutation rates. As such, biases in these polymerases are reflected in the library product (e.g. a preference for transition mutations versus transversions). An inherent limitation of this approach to library construction is a relative inability to affect the size of the gene being varied. Most DNA polymerases have extremely low rates of indel mutations (insertions or deletions), and most of these will result

in frameshift mutations in protein-coding regions, rendering members of the library unlikely to pass any downstream selection (See McInerney P, Adams P, and Hadi MZ. Mol Biol Int. 2014).

[0780] Additionally, biases in PCR and cloning can make it difficult to generate single libraries consisting of genes of different sizes. These limitations can severely limit the efficacy of directed evolution to enhance existing or engineer novel protein functions. In natural evolution, large changes in protein function or efficacy are typically associated with insertion and deletion mutations that are unlikely to occur during canonical library generation for mutagenesis. Furthermore, these mutations most commonly occur in regions of the protein in question that are predicted to form loops, as opposed to the hydrophobic core. Thus, most indels generated using a traditional unbiased approach are likely to either be deleterious or ineffective.

[0781] Libraries that could bias such mutations to the sites within the protein where they would be most likely to be beneficial, *e.g.*,. loop regions, would have a significant advantage over traditional libraries given that all libraries access only a fraction of the possible mutation space. Finally, although it is possible to generate genetic libraries with site-specific indel mutations through multistep PCR and clonal assembly using NNK primers or via DNA shuffling, these libraries cannot undergo additional rounds of 'indelgenesis' in continual evolution. Continuous evolution is a type of directed evolution with minimal user intervention. One such example is PACE (see Esvelt KM, Carlson JC, and Liu DR. Nature. 2011). Because continuous evolution occurs with minimal user intervention, any increase in library diversity during the evolution must occur using the native replication machinery. As such, although libraries of genes with inserted or removed codons as specific loci can be generated and screened in PACE, additional rounds of 'indelgenesis' are not possible.

[0782] It is envisioned that the programmability of prime editing (PE) can be leveraged to generate highly sophisticated, programmed genetic libraries for use in high-throughput screening and directed evolution (see FIG. 29A). PE can insert, change or remove defined numbers of nucleotides from specified genetic loci using information encoded in a prime editing guide RNA (PEgRNA) (see FIG. 29B). This enables the generation of targeted libraries with one or more amino acids inserted or removed from the loop regions wherein mutations are most likely to give rise to changes in function, without background introduction of nonfunctional frameshift mutations (see FIG. 29C). PE can be used to install specific sets

of mutations without regard for biases inherent in either DNA polymerase or the sequence being mutated.

[0783] For instance, while converting a CCC codon to a stop codon would be an unlikely occurrence via canonical library generation because it would require three consecutive mutations, including two transversions, PE could be used to convert any given, targeted codon to a TGA stop codon in one step. They could also be used to install programmed diversity at given positions, for instance by incorporating codons encoding any hydrophobic amino acid at a given site, while not encoding any others. Furthermore, because of the programmability of PE, multiple PEgRNAs could be utilized to generate multiple different edits at multiple sites simultaneously, enabling the generation of highly programmed libraries (see FIG. 29D). Additionally, it is possible to use reverse transcriptases with lower fidelity to generate regions of mutagenesis within an otherwise invariable library (such as the HIV-I reverse transcriptase or Bordetella phage reverse transcriptase) (see Naorem SS, Hin J, Wang S, Lee WR, Heng X, Miller JF, Guo H. Proc Natl Acad Sci 2017 and Martinez MA, Vartanian JP, Wain-Hobson S. Proc Natl Acad Sci USA 1994).

[0784] The possibility of iterative rounds of PE on the same site is also envisioned, allowing–for instance–the repeated insertion of codons at a single site, e.g., in a loop region. Also, it is envisioned that all of the above described approaches can be incorporated into continual evolution, enabling the generation of novel *in situ* evolving libraries (see FIG. 30). They could also be used to construct these libraries within other cell types where it would otherwise be difficult to assemble large libraries, for instance within mammalian cells. Generation of PE-encoding bacterial strains that have been optimized for directed evolution would be a useful additional tool for the identification of proteins and RNAs with improved or novel functionality. All of these uses of PE are non-obvious due to the novel nature of PEs. In conclusion, library generation via PE would be a highly useful tool in synthetic biology and directed evolution, as well as for high-throughput screening of protein and RNA combinatorial mutants.

Competing Approaches

[0785] The chief method by which diverse libraries are currently generated is by mutagenic PCR (see Cadwell RC and Joyce GF. PCR Methods Appl. 1992), described above. Insertions or deletions can be introduced via degenerated NNK primers at defined sites during PCR, although introducing such mutations at multiple sites requires multiple rounds of iterative PCR and cloning before constructing a more diverse library via mutagenic PCR, rendering

the method slow. An alternative, complementary method is DNA shuffling, where fragments of a library of genes generated via DNase treatment are introduced into a PCR reaction without primers, resulting in the annealing of different fragments to each other and the rapid generation of more diverse libraries than via mutagenic PCR alone (see Meyer AJ, Ellefson JW, Ellington AD. Curr Protoc Mol Biol. 2014). Although this approach can theoretically generate indel mutations, it more often results in frameshift mutations that destroy gene function. Furthermore, DNA shuffling requires a high degree of homology between gene fragments.

[0786] Both of these methods must be done *in vitro,* with the resulting library transformed into cells, while libraries generated by PE can be constructed in situ, enabling their use in continual evolution. While libraries can be constructed *in situ* through *in vivo* mutagenesis, these libraries rely on the host cellular machinery and exhibit biases against indels. Similarly, although traditional cloning methods can be used to generate site-specific mutational profiles, they cannot be used *in situ* and are generally assembled one at a time *in vitro* before being transformed into cells. The efficiency and broad functionality of PE in both prokaryotic and eukaryotic cell types further suggests that these libraries could be constructed directly in the cell type of interest, as opposed to being cloned into a model organism such as *E. coli* and then transferred into the cell or organism of interest. Another competing approach for targeted diversification is automated multiplex genome engineering, or MAGE, wherein multiple single-stranded DNA oligonucleotides can be incorporated within replication forks and result in programmable mutations[7]. However, MAGE requires significant modification of the host strain and can lead to a 100-fold increase in off-target or background mutations (see Nyerges Á et al. Proc Natl Acad Sci USA. 2016), whereas PE is more highly programmed and anticipated to result in fewer off-target effects. Additionally, MAGE has not been demonstrated in a wide variety of cell types, including mammalian cells.

[0787] By contrast, prime editing is a novel and non-obvious complementary technique for library generation.

Use of PE for Constructing Gene Libraries

[0788] PE may be used to construct gene libraries in a programmable manner.

[0789] In one example, PE can be used in a directed evolution experiment to introduce protein variants into gene libraries during a continual evolution experiment using PACE, permitting iterative accumulation of both point mutations and indels in a manner not possible via traditional approaches.

[0790] It has already been shown that PE can site-specifically and programmably insert nucleotides into a genetic sequence in *E. coli.* Directed evolution can be used to identify monobodies with improved binding to a specific epitope via a modified two-hybrid protein:protein binding PACE selection. Specific and highly variable loops within these monobodies contribute significantly to affinity and specificity. Improved monobody binding might be obtained rapidly in PACE by varying the length and composition of these loops in a targeted fashion. However, varying sequence length is not an established functionality of PACE. While library of varied loop sizes might be used as a starting point for PACE, no subsequent improvements to length would arise throughout the PACE selection, barring access to beneficial synergistic combinations of point mutations and indel mutations.

[0791] In various embodiments, PE can be used to improve the PACE selection by enabling the *in situ* generation and evolution of monobodies with varying loop lengths. To do so, the PACE *E. coli* strain may be introduced to an additional PE plasmid, which encodes the PE enzyme and one or more PEgRNAs. Expression of PE enzyme and PEgRNAs in the *E. coli* would be under the control of a small molecule delivered to the PACE lagoon at a rate selected by the experimenter.

[0792] In various embodiments, the PEgRNA components would contain a spacer directing the PE to the site of interest on the selection phage and would be designed such that a multiple of three nucleotides could be inserted at the target site such that a new PEgRNA binding site would be introduced, enabling the iterative insertion of one or more codons at the targeted site.

[0793] In parallel, another host *E. coli* strain might include PEgRNAs that would template the removal of one or more codons, enabling loop size to shrink during the evolution. A PACE experiment might utilize a mixture of both strains or alternate the two to permit the slow and controlled addition or removal of loop sequences.

[0794] In addition to the use of PE and PACE to create monobody libraries, this technique can also be applied to the evolution of antibodies using PE and PACE. The binding principles governing antibodies are very similar to those governing monobodies: the length of antibody complement-determining region loops is critical to their binding function. Further, longer loop lengths have been found to be critical in the development of rare antibodies with broadly protective activity against HIV-1 and other viral infections (see Mascola JR, Haynes BF. Immunol Rev. 2013). Application of PE as described above to an antibody or antibody-derived molecule would permit the generation of antibodies with diverse loop length and

varied loop sequence. In combination with PACE, such an approach would permit enhanced binding through loop geometries not accessible to standard PACE, and thus permit evolution of highly functional antibodies.

[0795] As a non-limiting example, the following PEgRNAs could be used to programmably modify the genome of a bacteriophage used in a continuous evolution experiment:

MODIFICATION	PEGRNA SEQUENCE
CCA INSERTION	UACACCAUCACGGUCUAUGCGUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUAUCUUCGCCCCAUGCAUAGACCGUGAU GG (SEQ ID NO: 101)
1 NT DELETION	CGCGUCGCGCUCGUCAGAUCGUUUUAGAGCUAGAAAUAGCAA GUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGC ACCGAGUCGGUGCUUUCCGCCUACCUGCAUCUGACGAGCGCG A (SEQ ID NO: 102)
POINT MUTATION	AUCGGAGAAUACAUGAACAUGUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCCGGAGAAUACAUGAACAUCGGACCCGCG CUAUCUUC (SEQ ID NO: 103)
NNN INSERTION (N=A/T/G/C)	UACACCAUCACGGUCUAUGCGUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCUAUCUUCGCCNNNUGCAUAGACCGUGAU GG (SEQ ID NO: 104)
ITERATIVE GGG INSERTION	UACACCAUCACGGUCUAUGCGUUUUAGAGCUAGAAAUAGCA AGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGCGGGGGGGGGGGGGUGCAUAGACCGUGA UGG (SEQ ID NO: 105)

[0796] In various embodiments, the use of PE for constructing gene libraries may make the use of the mutagenic activity of error-prone reverse transcriptases. The use of such mutagenic reverse transcriptase may facilitate the generation of mutagenized programmable libraries due to the lower fidelity of the error-prone RTs. As used herein, the term "error-prone" reverse transcriptase refers to a reverse transcriptase enzyme that occurs naturally or which has been derived from another reverse transcriptase (e.g., a wild type M-MLV reverse transcriptase) which has an error rate that is less than the error rate of wild type M-MLV reverse transcriptase. The error rate of wild type M-MLV reverse transcriptase is reported to be in the range of one error in 15,000 to 27,000 nucleobase incorporations. See Boutabout et al. (2001) "DNA synthesis fidelity by the reverse transcriptase of the yeast retrotransposon Ty1," *Nucleic Acids Res* 29(11):2217–2222, which is incorporated herein by reference.

[0797] Thus, for purposes of this application, the term "error prone" refers to those RT that have an error rate that is greater than one error in 15,000 nucleobase incorporation (6.7×10^{-5} or higher), e.g., 1 error in 14,000 nucleobases (7.14×10^{-5} or higher), 1 error in 13,000

nucleobases or fewer (7.7 x 10^{-5} or higher), 1 error in 12,000 nucleobases or fewer (7.7 x 10^{-5} or higher), 1 error in 11,000 nucleobases or fewer (9.1 x 10^{-5} or higher), 1 error in 10,000 nucleobases or fewer (1 x 10^{-4} or 0.0001 or higher), 1 error in 9,000 nucleobases or fewer (0.00011 or higher), 1 error in 8,000 nucleobases or fewer (0.00013 or higher) 1 error in 7,000 nucleobases or fewer (0.00014 or higher), 1 error in 6,000 nucleobases or fewer (0.00016 or higher), 1 error in 5,000 nucleobases or fewer (0.0002 or higher), 1 error in 4,000 nucleobases or fewer (0.00025 or higher), 1 error in 3,000 nucleobases or fewer (0.00033 or higher), 1 error in 2,000 nucleobase or fewer (0.00050 or higher), or 1 error in 1,000 nucleobases or fewer (0.001 or higher), or 1 error in 500 nucleobases or fewer (0.002 or higher), or 1 error in 250 nucleobases or fewer (0.004 or higher).

[0798] A variety of mutagenic RTs could be envisioned for generation of highly mutagenized programmable libraries. Two such examples are the mutagenic reverse transcriptases from *Bordetella* phage (see Handa, S., et al. *Nucl Acids Res* 9711-25 (2018), which is incorporated herein by reference) and *Legionella pneumophila* (see Arambula, D., et al. *Proc Natl Acad Sci USA* 8212-7 (2013), which is incorporated by reference). In the case of the RT from *Bordetella* phage (brt), an accessory protein might need to also be added (bavd) to Cas9 – or delivered *in trans* – as well as additional RNA sequences to the PEgRNA to improve binding of the mutagenic RT to the target site (see Handa, S., et al. *Nucl Acids Res* 9711-25 (2018)). When using mutagenic RTs, the template region of the PEgRNA might be enriched in adenosines or AAY codons to enhance diversity.

[0799] The amino acid sequence of the mutagenic RT from *Bordetella* phage is provided as follows. Like other RTs disclosed herein, the Brt protein may be fused to a napDNAbp as a fusion protein to form a functional PE.

Name	Sequence
brt mutagenic rt	MGKRHRNLIDQITTWENLLDAYRKTSHGKRRTWGYLEFKE YDLANLLALQAELKAGNYERGPYREFLVYEPKPRLISALEF KDRLVQHALCNIVAPIFEAGLLPYTYACRPDKGTHAGVCHV QAELRRTRATHFLKSDFSKFFPSIDRAALYAMIDKKIHCAAT RRLLRVVLPDEGVGIPIGSLTSQLFANVYGGAVDRLLHDELK QRHWARYMDDIVVLGDDPEELRAVFYRLRDFASERLGLKIS HWQVAPVSRGINFLGYRIWPTHKLLRKSSVKRAKRKVANFI

	KHGEDESLQRFLASWSGHAQWADTHNLFTWMEEQYGIAC H (SEQ ID NO: 129)

[0800] In the case of Brt from *Bodetella*, the PE fusion may also include an additional accessory protein (Bavd). The accessory protein may be fused to the PE fusion protein or provided *in trans*. The amino acid sequence of Bavd accessory protein is provided as follows:

Name	Sequence
bavd accessory protein to brt	MEPIEEATKCYDQMLIVERYERVISYLYPIAQSIPRKHGVARE MFLKCLLGQVELFIVAGKSNQVSKLYAADAG LAMLRFWLRFLAGIQKPHAMTPHQVETAQVLIAEVGRILGS WIARVNRKGQAGK (SEQ ID NO: 236)

[0801] In the case of Brt from *Bodetella*, the PEgRNA may comprise an additional nucleotide sequence added a PEgRNA, e.g., to the 5′ or 3′ end. Examplary sequence is as follows, which is originally from the *Bordetella phage* genome:

NAME	SEQUENCE
PEGRNA-ADDITION 1	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 237)

[0802] This PEgRNA addition sequence can be reduced in various ways to shorten the length. For example, the PEgRNA-addition 1 sequence could be reduced to the following exemplary alternative addition sequences:

NAME	SEQUENCE
PEGRNA-ADDITION 2	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUC (SEQ ID NO: 238)
PEGRNA-ADDITION 3	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCAAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 239)
PEGRNA-ADDITION 4	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGGUGGUCA (SEQ ID NO: 277)
PEGRNA-ADDITION 5	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAACACGUUACA

	CGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAGGCGGGGCCG ACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 240)
PEGRNA-ADDITION 6	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAGCCGGUUCUGUCCUGCUUCGGCGAACACGUUACA CGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAGGCGGGGCCG ACUUC (SEQ ID NO: 241)
PEGRNA-ADDITION 7	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCAAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 242)
PEGRNA-ADDITION 8	CAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGCCUUUCCUCA AUAGGUGGUCA (SEQ ID NO: 243)

[0803] In other embodiments, the PEgRNA addition sequence can be also be mutated. For example, the PEgRNA-addition 1 sequence could be mutated to the following exemplary alternative addition sequence:

NAME	SEQUENCE
PEGRNA-ADDITION 1 MUTATED	ACCUUCUUGCAUGGCUCUGCCAACGCUACGGCUUGGCGGGCUGGC CUUUCCUCAAUAGAUGAGCCGCCGGUUCUGUCCUGCUUCGGCGAA CACGUUACACGGUUCGGCAAAACGUCGAUUACUGAAAAUGGAAAG GCGGGGCCGACUUCAAGGGCAGGCUGGGAAAUAA (SEQ ID NO: 244)

[0804] In various embodiments relating to the use of PE for designing gene libraries, special PEgRNAs considerations may apply. For example, without wishing to be bound by theory, the additional PEgRNA sequences described above might be needed to enable efficient mutagenesis via mutagenic RTs. In another embodiment, iterative codon insertion using PE may required specific PEgRNA designs. For example, to insert a GGG (glycine) codon iteratively, the entire homology region of the PEgRNA might need to be composed of Gs, as shown above. This would mean that only certain sites could go iterative insertion. Additionally, the PAM sequence would not be able to be disrupted by the PE.

H. Use of prime editing for insertion of immunoepitopes

[0805] Prime editors may also be used as a means to insert known immunogenicity epitopes into endogenous or foreign genomic DNA, resulting in modification of the corresponding proteins for therapeutic or biotechnological applications (see FIGs. 31 and 32). Prior to the invention of prime editing, such insertions could be achieved only inefficiently and with high rates of indel formation from DSBs. prime editing solves the problem of high indel formation from insertion edits while generally offering higher efficiency than HDR. This lower rate of indel formation presents a major advantage of prime editing over HDR as a method for targeted DNA insertions, especially in the described application of inserting immunogenicity

epitopes. The length of epitopes is in a range from few bases to hundreds of bases. Prime editor is an efficient approach to achieve such targeted insertions in mammalian cells.

[0806] The key concept of the invention is the use of prime editors to insert a nucleotide sequence containing previously described immunogenicity epitopes into endogenous or foreign genomic DNA for the downregulation and/or destruction of their protein products and/or expressing cell types. Nucleotide sequences for immunogenic epitope insertion would be targeted to genes in a manner to produce fusion proteins of the targeted gene's coded protein and the inserted immunogenic epitope's corresponding protein translation. Patient's immune systems will have been previously trained to recognize these epitopes as a result of standard prior immunization from routine vaccination against, for example, tetanus or diphtheria or measles. As a result of the immunogenic nature of the fused epitopes, patient's immune systems would be expected to recognize and disable the prime edited protein (not just the inserted epitope) and potentially the cells from which it was expressed.

[0807] Precise genome targeting technologies using the CRISPR/Cas system have recently been explored in a wide range of applications, including the insertion of engineered DNA sequences into targeted genomic loci. Previously, homology-directed repair (HDR) has been used for this application, requiring an ssDNA donor template and repair initiation by means of a double-stranded DNA break (DSB). This strategy offers the broadest range of possible changes to be made in cells and is the only method available to insert large DNA sequences into mammalian cells. However, HDR is hampered by undesired cellular side effects stemming from its initiating DSB, such as high levels of indel formation, DNA translocations, large deletions, and P53 activation. In addition to these drawbacks, HDR is limited by low efficiency in many cell types (T cells are a notable exception to this observation). Recent efforts to overcome these drawbacks include fusing human Rad51 mutants to a Cas9 D10A nickase (RDN), resulting in a DSB-free HDR system that features improved HDR product:indel ratios and lower off target editing, but is still hampered by cell-type dependencies and only modest HDR editing efficiency.

[0808] Recently developed fusions of Cas9 to reverse transcriptases ("Prime editors") coupled with PEgRNAs represent a novel genome editing technology that offers a number of advantages over existing genome editing methods, including the ability to install any single nucleotide substitution, and to insert or delete any short stretch of nucleotides (up to at least several dozen bases) in a site-specific manner. Notably, PE edits are achieved with generally

low rates of unintended indels. As such, PE enables targeted insertion-based editing applications that have been previously impossible or impractical.

[0809] This particular aspect describes a method for using prime editing as a means to insert known immunogenicity epitopes into endogenous or foreign genomic DNA, resulting in modification of the corresponding proteins for therapeutic or biotechnological applications (see FIGs. 31 and 32). Prior to the invention of prime editing, such insertions could be achieved only inefficiently and with high rates of indel formation from DSBs. prime editing solves the problem of high indel formation from insertion edits while generally offering higher efficiency than HDR. This lower rate of indel formation presents a major advantage of prime editing over HDR as a method for targeted DNA insertions, especially in the described application of inserting immunogenicity epitopes. The length of epitopes is in a range from few bases to hundreds of bases. Prime editor is the most efficient and cleanest technology to achieve such targeted insertions in mammalian cells.

[0810] The key concept of this aspect is the use of prime editors to insert a nucleotide sequence containing previously described immunogenicity epitopes into endogenous or foreign genomic DNA for the downregulation and/or destruction of their protein products and/or expressing cell types. Nucleotide sequences for immunogenic epitope insertion would be targeted to genes in a manner to produce fusion proteins of the targeted gene's coded protein and the inserted immunogenic epitope's corresponding protein translation. Patient's immune systems will have been previously trained to recognize these epitopes as a result of standard prior immunization from routine vaccination against, for example, tetanus or diphtheria or measles. As a result of the immunogenic nature of the fused epitopes, patient's immune systems would be expected to recognize and disable the prime edited protein (not just the inserted epitope) and potentially the cells from which it was expressed.

[0811] Fusions to targeted genes would be engineered as needed to ensure the inserted epitope protein translation is exposed for immune system recognition. This could include targeted nucleotide insertions resulting in protein translations yielding C-terminal fusions of immunogenicity epitopes to targeted genes, N-terminal fusions of immunogenicity epitopes to targeted genes, or the insertion of nucleotides into genes so that immunogenicity epitopes are coded within surfaced-exposed regions of protein structure.

[0812] Protein linkers encoded as nucleotides inserted between the target gene sequence and the inserted immunogenicity epitope nucleotide sequence may need to be engineered as part of this invention to facilitate immune system recognition, cellular trafficking, protein

function, or protein folding of the targeted gene. These inserted nucleotide-encoded protein linkers may include (but are not limited to) variable lengths and sequences of the XTEN linker or variable lengths and sequences of Glycine-Serine linkers. These engineered linkers have been previous used to successfully facilitate protein fusions. Examplary linkers may include any of those described herein, including the amino acid sequence (GGGGS)n (SEQ ID NO: 165), (G)n (SEQ ID NO: 166), (EAAAK)n (SEQ ID NO: 167), (GGS)n (SEQ ID NO: 168), (SGGS)n (SEQ ID NO: 169), (XP)n (SEQ ID NO: 170), or any combination thereof, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, the linker comprises the amino acid sequence (GGS)n (SEQ ID NO: 176), wherein n is 1, 3, or 7. In some embodiments, the linker comprises the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 171). In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 172). In some embodiments, the linker comprises the amino acid sequence SGGSGGSGGS (SEQ ID NO: 173). In some embodiments, the linker comprises the amino acid sequence SGGS (SEQ ID NO: 174).

[0813] Distinguishing features of this aspect include the ability to use previously acquired immune responses to specific amino acid sequences as a means to induce an immune response against otherwise non-immunogenic proteins. Another distinguishing feature is the ability to insert the nucleotide sequences of these immunogenic epitopes in a targets manner that does not induce high levels of unwanted indels as a by-product editing and is efficient in its insertion. This specific application of PE has the ability to combine cell type-specific delivery methods (such as AAV serotypes) to insert epitopes in cell types that are of interest to trigger an immune response to.

[0814] Prime editing as a means of inserting immunogenic epitopes into pathogenic genes could be used to program the patient's immune system to fight a wide variety of diseases (not limited to cancer as with immuno-oncology strategies). An immediately relevant use of this technology would be as a cancer therapeutic as it could undermine a tumor's immune escape mechanism by causing an immune response to a relevant oncogene like HER2 or growth factors like EGFR. Such an approach could seem similar to T-cell engineering, but one novel advance of this approach is that it can be utilized in many cell types and for diseases beyond cancer, without needed to generate and introduce engineered T-cells into patients.

[0815] Using PE to insert an immunogenicity epitope which most people are already vaccinated against (tetanus, pertussis, diphtheria, measles, mumps, rubella, etc.) into a foreign

or endogenous gene that drives a disease, so the patient's immune system learns to disable that protein.

[0816] Diseases that stand to have a potential therapeutic benefit from the aforementioned strategy include those caused by aggregation of toxic proteins, such as in fatal familial insomnia. Other diseases that could benefit include those caused by pathogenic overexpression of an otherwise nontoxic endogenous protein, and those caused by foreign pathogens.

[0817] Primary therapeutic indications include those mentioned above such as therapeutics for cancer, prion and other neurodegenerative diseases, infectious diseases, and preventative medicine. Secondary therapeutic indications may include preventative care for patients with late-onset genetic diseases. It is expected that current standard of care medicines may be used in conjunction with prime editing for some diseases, like particularly aggressive cancers, or in cases where medications help alleviate disease symptoms until the disease completely cured. Below are examples of immunoepitopes that may be inserted into genes by the herein disclosed prime editors:

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
1	TETANUS TOXOID	QYIKANSKFIGITEL (SEQ ID NO: 396)	CATGATATAAAAGCAAATTCTAAATTTATAGGTATAACTGAACTA (SEQ ID NO: 397)

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
2	DIPHTHERIA TOXIN MUTANT CRM197	GADDVVDSSKSF VMENFSSYHGTK PGYVDSIQKGIQK PKSGTQGNYDDD WKEFYSTDNKYD AAGYSVDNENPL SGKAGGVVKVTY PGLTKVLALKVD NAETIKKELGLSL TEPLMEQVGTEEF IKRFGDGASRVVL SLPFAEGSSSVEYI NNWEQAKALSVE LEINFETRGKRGQ DAMYEYMAQAC AGNRVRRSVGSSL SCINLDWDVIRDK TKTKIESLKEHGPI KNKMSESPNKTV SEEKAKQYLEEFH QTALEHPELSELK TVTGTNPVFAGA NYAAWAVNVAQV IDSETADNLEKTT AALSILPGIGSVM GIADGAVHHNTEE IVAQSIALSSLMVA QAIPLVGELVDIGF AAYNFVESIINLFQ VVHNSYNRPAYSP GHKTQPFLHDGY AVSWNTVEDSIIR TGFQGESGHDIKI TAENTPLPIAGVL LPTIPGKLDVNKS KTHISVNGRKIRM RCRAIDGDVTFCR PKSPVYVGNGVH ANLHVAFHRSSSE KIHSNEISSDSIGV LGYQKTVDHTKV NSKLSLFFEIKS (SEQ ID NO: 398)	GGCGCCGACGACGTGGTGGACAGCAG CAAGAGCTTCGTGATGGAGAACTTCA GCAGCTACCACGGCACCAAGCCCGGC TACGTGGACAGCATCCAGAAGGGCAT CCAGAAGCCCAAGAGCGGCACCCAGG GCAACTACGACGACGACTGGAAGGAG TTCTACAGCACCGACAACAAGTACGA CGCCGCCGGCTACAGCGTGGACAACG AGAACCCCCTGAGCGGCAAGGCCGGC GGCGTGGTGAAGGTGACCTACCCCGG CCTGACCAAGGTGCTGGCCCTGAAGG TGGACAACGCCGAGACCATCAAGAAG GAGCTGGGCCTGAGCCTGACCGAGCC CCTGATGGAGCAGGTGGGCACCGAGG AGTTCATCAAGAGGTTCGGCGACGGC GCCAGCAGGGTGGTGCTGAGCCTGCC CTTCGCCGAGGGCAGCAGCAGCGTGG AGTACATCAACAACTGGGAGCAGGCC AAGGCCCTGAGCGTGGAGCTGGAGAT CAACTTCGAGACCAGGGGCAAGAGGG GCCAGGACGCCATGTACGAGTACATGG CCCAGGCCTGCGCCGGCAACAGGGTG AGGAGGAGCGTGGGCAGCAGCCTGAG CTGCATCAACCTGGACTGGGACGTGAT CAGGGACAAGACCAAGACCAAGATCG AGAGCCTGAAGGAGCACGGCCCCATC AAGAACAAGATGAGCGAGAGCCCCAA CAAGACCGTGAGCGAGGAGAAGGCC AAGCAGTACCTGGAGGAGTTCCACCA GACCGCCCTGGAGCACCCCGAGCTGA GCGAGCTGAAGACCGTGACCGGCACC AACCCCGTGTTCGCCGGCGCCAACTA CGCCGCCTGGGCCGTGAACGTGGCCC AGGTGATCGACAGCGAGACCGCCGAC AACCTGGAGAAGACCACCGCCGCCCT GAGCATCCTGCCCGGCATCGGCAGCGT GATGGGCATCGCCGACGGCGCCGTGC ACCACAACACCGAGGAGATCGTGGCC CAGAGCATCGCCCTGAGCAGCCTGAT GGTGGCCCAGGCCATCCCCCTGGTGG GCGAGCTGGTGGACATCGGCTTCGCC GCCTACAACTTCGTGGAGAGCATCATC AACCTGTTCCAGGTGGTGCACAACAG CTACAACAGGCCCGCCTACAGCCCCG GCCACAAGACCCAGCCCTTCCTGCAC

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
			GACGGCTACGCCGTGAGCTGGAACAC CGTGGAGGACAGCATCATCAGGACCG GCTTCCAGGGCGAGAGCGGCCACGAC ATCAAGATCACCGCCGAGAACACCCC CCTGCCCATCGCCGGCGTGCTGCTGCC CACCATCCCCGGCAAGCTGGACGTGA ACAAGAGCAAGACCCACATCAGCGTG AACGGCAGGAAGATCAGGATGAGGTG CAGGGCCATCGACGGCGACGTGACCT TCTGCAGGCCCAAGAGCCCCGTGTAC GTGGGCAACGGCGTGCACGCCAACCT GCACGTGGCCTTCCACAGGAGCAGCA GCGAGAAGATCCACAGCAACGAGATC AGCAGCGACAGCATCGGCGTGCTGGG CTACCAGAAGACCGTGGACCACACCA AGGTGAACAGCAAGCTGAGCCTGTTC TTCGAGATCAAGAGC (SEQ ID NO: 399)
3	MUMPS IMMUNOEPITOPE 1	GTYRLIPNARANLTA (SEQ ID NO: 400)	GGCACCTACAGGCTGATCCCCAACGC CAGGGCCAACCTGACCGCC (SEQ ID NO: 401)
4	MUMPS IMMUNOEPITOPE 2 MUMPS IMMUNOEPITOPE 1	PSKFFTISDSATFAPGPVSNA (SEQ ID NO: 402) PSKLFIMLDNATFAPGPVVNA (SEQ ID NO: 404)	CCGAGCAAATTTTTTACCATTAGCGAT AGCGCGACCTTTGCGCCGGGCCCGGT GAGCAACGCG (SEQ ID NO: 403) CCGAGCAAACTGTTTATTATGCTGGAT AACGCGACCTTTGCGCCGGGCCCGGT GGTGAACGCG (SEQ ID NO: 405) SELECTED EXAMPLES FROM HEMAGGLUTININ–NEURAMINIDASE (HN) DIVERSITY AMONG OUTBREAK STRAINS (TABLE1) DIVERGENCE BETWEEN VACCINE STRAIN JL5 AND OUTBREAK STRAINS (TABLE2)
5	RUBELLA VIRUS (RV)	TPPPYQVSCGGESDRASARVIDPAAQS (SEQ ID NO: 406)	ACCCCCCCCCCCTACCAGGTGAGCTGC GGCGGCGAGAGCGACAGGGCCAGCG CCAGGGTGATCGACCCCGCCGCCCAG AGC (SEQ ID NO: 407)

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
6	HEMAGGLUTININ	PEYAYKIVKNKK MEDGFLQGMVD GWYGHHSNEQGS GLMENERTLDKA NPNNDLCSWSDH EASSNNTNQEDLL QRESRRKKRIGTS TLNQRGNCNTKC QTEEARLKREEVS LVKSDQCSNGSLQ CRANNSTEQVD (SEQ ID NO: 408)	CCCGAGTACGCCTACAAGATCGTGAA GAACAAGAAGATGGAGGACGGCTTCC TGCAGGGCATGGTGGACGGCTGGTAC GGCCACCACAGCAACGAGCAGGGCAG CGGCCTGATGGAGAACGAGAGGACCC TGGACAAGGCCAACCCCAACAACGAC CTGTGCAGCTGGAGCGACCACGAGGC CAGCAGCAACAACACCAACCAGGAGG ACCTGCTGCAGAGGGAGAGCAGGAGG AAGAAGAGGATCGGCACCAGCACCCT GAACCAGAGGGGCAACTGCAACACCA AGTGCCAGACCGAGGAGGCCAGGCTG AAGAGGGAGGAGGTGAGCCTGGTGA AGAGCGACCAGTGCAGCAACGGCAGC CTGCAGTGCAGGGCCAACAACAGCAC CGAGCAGGTGGAC (SEQ ID NO: 409)
7	NEURAMINIDASE	TKSTNSRSGGISG PDNEAPVGEAPSP YGDNPRPNDGNN IRIGSKGYNGIITD TIEESCSCYPDAK VVKSVELDSTIWT SGSSPNQKIITIGW DPNGWTGTPMSP NGAYGTDGPSNG QANQHQAESISA GNSSLCPIRDNWH GSNRSWSWPDGA E (SEQ ID NO: 410)	ACCAAGAGCACCAACAGCAGGAGCG GCGGCATCAGCGGCCCCGACAACGAG GCCCCCGTGGGCGAGGCCCCCAGCCC CTACGGCGACAACCCCAGGCCCAACG ACGGCAACAACATCAGGATCGGCAGC AAGGGCTACAACGGCATCATCACCGA CACCATCGAGGAGAGCTGCAGCTGCT ACCCCGACGCCAAGGTGGTGAAGAGC GTGGAGCTGGACAGCACCATCTGGAC CAGCGGCAGCAGCCCCAACCAGAAGA TCATCACCATCGGCTGGGACCCCAACG GCTGGACCGGCACCCCCATGAGCCCC AACGGCGCCTACGGCACCGACGGCCC CAGCAACGGCCAGGCCAACCAGCACC AGGCCGAGAGCATCAGCGCCGGCAAC AGCAGCCTGTGCCCCATCAGGGACAA CTGGCACGGCAGCAACAGGAGCTGGA GCTGGCCCGACGGCGCCGAG (SEQ ID NO: 411)
8	TAP1 (TRANSPORT ANTIGEN PRESENTATION) ON H5N1 VIRUS	EKIVLLLAMMEKI VLLLAKCQTPMG AIKAVDGVTNKCP YLGSPSF (SEQ ID NO: 412)	GAGAAGATCGTGCTGCTGCTGGCCATG ATGGAGAAGATCGTGCTGCTGCTGGCC AAGTGCCAGACCCCCATGGGCGCCAT CAAGGCCGTGGACGGCGTGACCAACA AGTGCCCCTACCTGGGCAGCCCCAGC TTC (SEQ ID NO: 413)

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
	HEMAGGLUTININ		
9	TAP2 (TRANSPORT ANTIGEN PRESENTATION) ON H5N1 VIRUS NEURAMINIDASE	IRPCFWVELNPNQKIITIRPCFWVELICYPDAGEIT (SEQ ID NO: 414)	ATCAGGCCCTGCTTCTGGGTGGAGCTGAACCCCAACCAGAAGATCATCACCATCAGGCCCTGCTTCTGGGTGGAGCTGATCTGCTACCCCGACGCCGGCGAGATCACC (SEQ ID NO: 415)
10	HEMAGGLUTININ EPITOPES TOWARD CLASS I HLA	MEKIVLLLAEKIVLLLAMCPYLGSPSFKCQTPMGAIKAVDGVTNK (SEQ ID NO: 416)	ATGGAGAAGATCGTGCTGCTGCTGGCCGAGAAGATCGTGCTGCTGCTGGCCATGTGCCCCTACCTGGGCAGCCCCAGCTTCAAGTGCCAGACCCCCATGGGCGCCATCAAGGCCGTGGACGGCGTGACCAACAAG (SEQ ID NO: 417)
11	NEURAMINIDASE EPITOPES TOWARD CLASS I HLA	NPNQKIITICYPDAGEITIRPCFWVELRPCFWVELI (SEQ ID NO: 418)	AACCCCAACCAGAAGATCATCACCATCTGCTACCCCGACGCCGGCGAGATCACCATCAGGCCCTGCTTCTGGGTGGAGCTGAGGCCCTGCTTCTGGGTGGAGCTGATC (SEQ ID NO: 419)
12	HEMAGGLUTININ EPITOPES TOWARD CLASS II HLA	MVSLVKSDQIGTSTLNQR (SEQ ID NO: 420)	ATGGTGAGCCTGGTGAAGAGCGACCAGATCGGCACCAGCACCCTGAACCAGAGG (SEQ ID NO: 421)
13	NEURAMINIDASE EPITOPES TOWARD CLASS II HLA	YNGIITDTI (SEQ ID NO: 422)	TACAACGGCATCATCACCGACACCATC (SEQ ID NO: 423)
14	HEMAGGLUTININ EPITOPE H5N1-	MEKIVLLLAEKIVLLLAMMVSLVKSDQCPYLGSPSFIGTSTLNQRKCQTPMGAIK	ATGGAGAAGATCGTGCTGCTGCTGGCCGAGAAGATCGTGCTGCTGCTGGCCATGATGGTGAGCCTGGTGAAGAGCGACCAGTGCCCCTACCTGGGCAGCCCCAGCTTCA

VACCINE	DISEASE	EPITOPE AMINO ACID SEQUENCE	EXAMPLE NUCLEIC ACID SEQUENCE (8)
	BOUND CLASS I AND CLASS II HLA	AVDGVTNK (SEQ ID NO: 424)	TCGGCACCAGCACCCTGAACCAGAGG (SEQ ID NO: 425)
15	NEURAMINIDASE EPITOPE H5N1-BOUND CLASS I AND CLASS II HLA	NPNQKIITIYNGIIT DTICYPDAGEITIRP CFWVELRPCFWVE LI (SEQ ID NO: 426)	AACCCCAACCAGAAGATCATCACCATCT ACAACGGCATCATCACCGACACCATCTG CTACCCCGACGCCGGCGAGATCACCATC AGGCCCTGCTTCTGGGTGGAGCTGAGG CCCTGCTTCTGGGTGGAGCTGATC (SEQ ID NO: 427)

[0818] Additional immunoepitopes may also be installed which are known in the art. Any of the immunoepitopes available from the Immune Epitope Database and Analysis Resource (iedb.org/epitopedetails_v3.php) (the contents of which are incorporated herein by reference) may be installed by the prime editors disclosed herein.

[0819] In some embodiments, the immunoepitopes which may be installed by the prime editors disclosed herein may include any of the following epitopes:

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1	TETANUS TOXOID	QYIKANSKFIGITEL	396	NA
2	DIPHTHERIA TOXIN MUTANT CRM197	GADDVVDSSKSFVMENFSSYHGTKP GYVDSIQKGIQKPKSGTQGNYDDDW KEFYSTDNKYDAAGYSVDNENPLSG KAGGVVKVTYPGLTKVLALKVDNA ETIKKELGLSLTEPLMEQVGTEEFIKR FGDGASRVVLSLPFAEGSSSVEYINN WEQAKALSVELEINFETRGKRGQDA MYEYMAQACAGNRVRRSVGSSLSCI NLDWDVIRDKTKTKIESLKEHGPIKN KMSESPNKTVSEEKAKQYLEEFHQT ALEHPELSELKTVTGTNPVFAGANYA AWAVNVAQVIDSETADNLEKTTAALS ILPGIGSVMGIADGAVHHNTEEIVAQS IALSSLMVAQAIPLVGELVDIGFAAYN FVESIINLFQVVHNSYNRPAYSPGHKT QPFLHDGYAVSWNTVEDSIIRTGFQG	428	NA

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
		ESGHDIKITAENTPLPIAGVLLPTIPGK LDVNKSKTHISVNGRKIRMRCRAIDG DVTFCRPKSPVYVGNGVHANLHVAF HRSSSEKIHSNEISSDSIGVLGYQKTV DHTKVNSKLSLFFEIKS		
3	MUMPS	GTYRLIPNARANLTA	SEQ ID NO: 400	NA
4	MUMPS	PSKFFTISDSATFAPGPVSNA; PSKLFIMLDNATFAPGPVVNA	402; 404	NA
5	RUBELLA VIRUS (RV)	TPPPYQVSCGGESDRASARVIDPAAQ S	406	NA
6	HEMAGGLUTININ	PEYAYKIVKNKKMEDGFLQGMVDG WYGHHSNEQGSGLMENERTLDKAN PNNDLCSWSDHEASSNNTNQEDLLQ RESRRKKRIGTSTLNQRGNCNTKCQT EEARLKREEVSLVKSDQCSNGSLQCR ANNSTEQVD	408	NA
7	NEURAMINIDASE	TKSTNSRSGGISGPDNEAPVGEAPSP YGDNPRPNDGNNIRIGSKGYNGIITD TIEESCSCYPDAKVVKSVELDSTIWTS GSSPNQKIITIGWDPNGWTGTPMSPN GAYGTDGPSNGQANQHQAESISAGN SSLCPIRDNWHGSNRSWSWPDGAE	410	NA
8	TAP (TRANSPORT ANTIGEN PRESENTATION) ON H5N1 VIRUS HEMAGGLUTININ	EKIVLLLAMMEKIVLLLAKCQTPMG AIKAVDGVTNKCPYLGSPSF	412	NA
9	TAP (TRANSPORT ANTIGEN PRESENTATION) ON H5N1 VIRUS NEURAMINIDASE	IRPCFWVELNPNQKIITIRPCFWVELIC YPDAGEIT	414	NA
10	HEMAGGLUTININ EPITOPES TOWARD CLASS I HLA	MEKIVLLLAEKIVLLLAMCPYLGSPS FKCQTPMGAIKAVDGVTNK	416	NA
11	NEURAMINIDASE EPITOPES TOWARD CLASS I HLA	NPNQKIITICYPDAGEITIRPCFWVELR PCFWVELI	418	NA

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
12	HEMAGGLUTININ EPITOPES TOWARD CLASS II HLA	MVSLVKSDQIGTSTLNQR	420	NA
13	NEURAMINIDASE EPITOPES TOWARD CLASS II HLA	YNGIITDTI	422	NA
14	HEMAGGLUTININ EPITOPE H5N1-BOUND CLASS I AND CLASS II HLA	MEKIVLLLAEKIVLLLAMMVSLVKSDQCPYLGSPSFIGTSTLNQRKCQTPMGAIKAVDGVTNK	424	NA
15	NEURAMINIDASE EPITOPE H5N1-BOUND CLASS I AND CLASS II HLA	NPNQKIITIYNGIITDTICYPDAGEITIRPCFWVELRPCFWVELI	426	NA
16	CORYNEBACTERIUM DIPHTHERIAE	AACAGNRVRRSVGSSLKC	899	SRC280292
17	MEASLES VIRUS STRAIN EDMONSTON	AADHCPVVEVNGVTI	900	P69353.1
18	MEASLES VIRUS STRAIN EDMONSTON	AAHLPTGTPLDID	901	P04851.1
19	BORDETELLA PERTUSSIS	AALAVWAGLAVQ	902	Q00879.1
20	MEASLES VIRUS STRAIN EDMONSTON	AALGVATAAQITAGI	903	P69353.1
21	RUBELLA VIRUS STRAIN THERIEN	AALLNTPPPYQVSCGGESDRATAR	904	P07566.1
22	RUBELLA VIRUS	AAQSFTGVVYGTHTT	905	BAA28178.1
23	RUBELLA VIRUS	ACEVEPAFGHSDAAC	906	BAA28178.1
24	RUBELLA VIRUS	ACTFWAVNAYSSGGY	907	BAA28178.1
25	RUBELLA VIRUS	ADDPLLR	908	BAA19893.1
26	RUBELLA VIRUS	ADDPLLRT	909	CAJ88851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
27	MEASLES VIRUS STRAIN EDMONSTON	AEMICDIDTYIVEAG	910	P04851.1
28	MEASLES VIRUS STRAIN EDMONSTON	AEMICDIDTYIVEAGLASFI	911	P04851.1
29	MEASLES VIRUS STRAIN EDMONSTON	AEPLLSC	912	P04851.1
30	BORDETELLA PERTUSSIS	AFVSTSSSRRYTEVY	913	CAD44970.1
31	BORDETELLA PERTUSSIS	AGFIYRETFCITTIYKTGQPAADHYYSKVTA	914	P04979.1
32	RUBELLA VIRUS	AGLLACCAKCLYYLR	915	BAA28178.1
33	RUBELLA VIRUS	AHTTSDPWHPPG	916	BAA19893.1
34	MEASLES VIRUS STRAIN EDMONSTON	AIAKLEDAKELLESS	917	P69353.1
35	MEASLES VIRUS STRAIN EDMONSTON	AIDNLRASLETTNQA	918	P69353.1
36	BORDETELLA PERTUSSIS	AKGVEFR	919	ACI16088.1
37	MEASLES VIRUS STRAIN EDMONSTON	AKWAVPTTRTDDKLR	920	P08362.1
38	MEASLES VIRUS STRAIN EDMONSTON	ALAEVLKKPV	921	ABO69699.1
39	MEASLES VIRUS STRAIN EDMONSTON	ALGVINTLEWIPRFK	922	P08362.1
40	MEASLES VIRUS STRAIN EDMONSTON	ALHQSMLNSQAIDNL	923	P69353.1
41	MEASLES VIRUS STRAIN EDMONSTON	ALIGILSLFV	924	ABI54110.1
42	RUBELLA VIRUS	ALLNTPPPYQVSCGGESDRA	925	CAJ88851.1
43	RUBELLA VIRUS STRAIN M33	ALLNTPPPYQVSCGGESDRASARV	926	CAJ88851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
44	RUBELLA VIRUS	ALVEGLAPGGGNCHL	927	BAA28178.1
45	BORDETELLA PERTUSSIS	AMAAWSERAGEA	928	P04977.1
46	MEASLES VIRUS STRAIN EDMONSTON	ANCASILCKCYTTGT	929	P69353.1
47	BORDETELLA PERTUSSIS	ANPNPYTSRRSV	930	P04977.1
48	RUBELLA VIRUS	APGPGEVW	931	CAJ88851.1
49	RUBELLA VIRUS	APLPPHTTERIETRSARHPWRIR	932	ABD64214.1
50	RUBELLA VIRUS VACCINE STRAIN RA27/3	APPMPPQPPRAHGQHYGHHHHQLPFLG	933	CAA33016.1
51	RUBELLA VIRUS STRAIN THERIEN	APPTLPQPPCAHGQHYGHHHHQLPFLG	934	P07566.1
52	RUBELLA VIRUS	APPTLPQPPRAHGQHYGHHHHQLPFLG	935	ABD64214.1
53	BORDETELLA PERTUSSIS	APQPGPQPPQPPQPQPEAPAPQ	936	P14283.3
54	RUBELLA VIRUS	AQLASYFNPGGSYYK	937	BAA28178.1
55	MEASLES VIRUS STRAIN EDMONSTON	ARAAHLPTGTPLD	938	P04851.1
56	MEASLES VIRUS STRAIN EDMONSTON	ARLVSEIAMHTTEDK	939	P04851.1
57	MEASLES VIRUS STRAIN EDMONSTON	ARLVSEIAMHTTEDKISRAV	940	P04851.1
58	MEASLES VIRUS STRAIN EDMONSTON-B	ASDVETAEGGEIHELLR	941	P03422.1
59	MEASLES MORBILLIVIRUS	ASDVETAEGGEIHELLRLQ	942	ABO69699.1
60	MEASLES VIRUS STRAIN EDMONSTON-B	ASDVETAEGGEIHELLRLQSR	943	P03422.1
61	MEASLES MORBILLIVIRUS	ASDVETAEGGEIHKLLRLQ	944	AAA63285.1
62	RUBELLA VIRUS STRAIN M33	ASDVLPGHWLQG	945	NP_740663.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
63	MEASLES VIRUS STRAIN EDMONSTON	ASELGITAEDARLVS	946	P04851.1
64	MEASLES VIRUS STRAIN EDMONSTON	ASELGITAEDARLVSEIAMH	947	P04851.1
65	MEASLES VIRUS STRAIN EDMONSTON	ASESSQDPQDSRR	948	P04851.1
66	MEASLES VIRUS STRAIN EDMONSTON	ASILCKCYTTGTIIN	949	P69353.1
67	RUBELLA VIRUS	ASPVCQRHSPDCSRL	950	BAA28178.1
68	BORDETELLA PERTUSSIS	ASQARWTGATRA	951	BAF35031.1
69	RUBELLA VIRUS STRAIN THERIEN	ASYFNPGGSYYKQYHPTACEVEPAFGHS	952	P07566.1
70	MEASLES VIRUS STRAIN EDMONSTON	ASYKVMTRSSHQSLV	953	P69353.1
71	BORDETELLA PERTUSSIS	ASYVKKPKEDVD	954	ACI16088.1
72	MEASLES VIRUS STRAIN EDMONSTON	ATAAQITAGIALHQS	955	P69353.1
73	RUBELLA VIRUS	ATPERPRL	956	CAJ88851.1
74	MEASLES VIRUS STRAIN EDMONSTON	AVCLGGLIGIPALIC	957	P69353.1
75	MEASLES VIRUS STRAIN EDMONSTON	AVGPRQAQVSF	958	P04851.1
76	RUBELLA VIRUS	AVNAYSSGGYAQLAS	959	BAA28178.1
77	RUBELLA VIRUS	AVSETRQTWAEWAAA	960	BAA28178.1
78	MEASLES VIRUS STRAIN EDMONSTON	AVTAPDTAADSELRR	961	P04851.1
79	MEASLES VIRUS STRAIN EDMONSTON	AVTAPDTAADSELRRWIKYT	962	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
80	BORDETELLA PERTUSSIS	AYGGIIKDAPPGAGFIYRETFC	963	P04979.1
81	RUBELLA VIRUS	CALPLAGLLACCAKC	964	BAA28178.1
82	RUBELLA VIRUS	CARIWNGTQRACTFW	965	BAA28178.1
83	MEASLES VIRUS STRAIN EDMONSTON	CARTLVSGSFGNRFI	966	P69353.1
84	BORDETELLA PERTUSSIS	CASPYEGRYRDMYDALRBRLLY	967	SRC280066
85	BORDETELLA PERTUSSIS	CAVFVRSGQPVIGA	968	AAA83981.1
86	RUBELLA VIRUS	CCAKCLYYLRGAIAPR	969	BAA28178.1
87	MEASLES VIRUS STRAIN EDMONSTON	CCRGRCNKKGEQVGM	970	P69353.1
88	RUBELLA VIRUS	CEIPTDVSCEGLGAW	971	BAA28178.1
89	BORDETELLA PERTUSSIS	CFGKDLKRPGSSPMEV	972	P0A3R5.1
90	MEASLES MORBILLIVIRUS	CFQQACKGKIQALCE	973	P06830.1
91	MEASLES MORBILLIVIRUS	CFQQACKGKIQALCENPEWAPLKDNRIPS	974	AAR89413.1
92	RUBELLA VIRUS	CGGESDRASARVIDP	975	BAA28178.1
93	BORDETELLA PERTUSSIS	CITTIYKTGQPAADHYYSKVTA	976	P04979.1
94	MEASLES MORBILLIVIRUS	CKGKIQALCENPEWA	977	AAR89413.1
95	MEASLES VIRUS STRAIN EDMONSTON	CKPWQESRKNKAQ	978	P04851.1
96	MEASLES VIRUS STRAIN EDMONSTON	CNKKGEQVGMSRPGL	979	P69353.1
97	RUBELLA VIRUS	CNVTTEHPFCNTPHG	980	BAA28178.1
98	BORDETELLA PERTUSSIS	CQVGSSNSAF	981	P04977.1
99	MEASLES MORBILLIVIRUS	CSGPTTIRGQFS	982	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
100	BORDETELLA PERTUSSIS	CTSPYDGKYWSMYSRL	983	AAA83981.1
101	MEASLES VIRUS STRAIN EDMONSTON	CVLADSESGGHITHS	984	P08362.1
102	MEASLES MORBILLIVIRUS	CYTTGTIINQDPDKILTYIAADHC	985	AAF02706.1
103	RUBELLA VIRUS	DADDPLLR	986	CAJ88851.1
104	MEASLES VIRUS STRAIN EDMONSTON	DARAAHLPTGTPLDI	987	P04851.1
105	MEASLES VIRUS STRAIN EDMONSTON	DARAAHLPTGTPLDIDTASE	988	P04851.1
106	MEASLES VIRUS STRAIN EDMONSTON	DCHAPTYLPAEVDGD	989	P08362.1
107	RUBELLA VIRUS	DCSRLVGATPERPRL	990	BAA28178.1
108	MEASLES VIRUS STRAIN EDMONSTON	DDKLRMETCFQQACK	991	P08362.1
109	RUBELLA VIRUS	DDPLLRTA	992	CAJ88851.1
110	RUBELLA VIRUS	DDPLLRTAPGPGEVW	993	BAA28178.1
111	BORDETELLA PERTUSSIS	DDPPATVYRYD	994	P04977.1
112	BORDETELLA PERTUSSIS	DDPPATVYRYDSRPPED	995	CAD44970.1
113	BORDETELLA PERTUSSIS	DDPPATVYRYDSRPPEDV	996	ACI04548.1
114	MEASLES MORBILLIVIRUS	DEVGLRTPQRFTDLV	997	P06830.1
115	MEASLES VIRUS STRAIN EDMONSTON	DHCPVVEVNGVTIQV	998	P69353.1
116	MEASLES VIRUS STRAIN EDMONSTON	DIDTASESSQDPQ	999	P04851.1
117	MEASLES VIRUS STRAIN EDMONSTON	DINKVLEKLGYSGGD	1000	P69353.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
118	BORDETELLA PERTUSSIS	DLIAYKQ	1001	ACI16088.1
119	MEASLES VIRUS STRAIN EDMONSTON	DLIGQKLGLKLLRYY	1002	P69353.1
120	RUBELLA VIRUS	DLQKALEAQSRALRAELAA	1003	P07566.1
121	MEASLES MORBILLIVIRUS	DLQYVLATYDTSRVE	1004	P06830.1
122	MEASLES VIRUS STRAIN EDMONSTON	DLSLRRFMV	1005	P04851.1
123	MEASLES VIRUS STRAIN EDMONSTON	DLSNCMVALGELKLA	1006	P08362.1
124	RUBELLA VIRUS	DLVEYIMNYTGNQQSRWGLGSPNC	1007	CAJ88851.1
125	MEASLES MORBILLIVIRUS	DLVKFISDKIKFLNP	1008	AAR89413.1
126	MEASLES MORBILLIVIRUS	DLVKFISTKIKFLNP	1009	SRC280117
127	MEASLES VIRUS STRAIN EDMONSTON	DLYKSNHNNV	1010	P08362.1
128	BORDETELLA PERTUSSIS	DNVLDHLTGR	1011	ACI04548.1
129	BORDETELLA PERTUSSIS	DNVLDHLTGRSC	1012	P04977.1
130	BORDETELLA PERTUSSIS	DNVLDHLTGRSCQ	1013	P04977.1
131	MEASLES MORBILLIVIRUS	DPDKILTYIAA	1014	AAF02706.1
132	RUBELLA VIRUS STRAIN THERIEN	DPGDLVEYIMNYTGNQQSR	1015	P07566.1
133	RUBELLA VIRUS	DPLLRTAP	1016	CAJ88851.1
134	RUBELLA VIRUS	DPLLRTAPGPGEVWVTPVIGSQ	1017	CAJ88851.1
135	MEASLES VIRUS STRAIN EDMONSTON	DPQDSRRSAEPLL	1018	P04851.1
136	MEASLES VIRUS STRAIN EDMONSTON	DPVIDRLYLSSHRGV	1019	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
137	MEASLES VIRUS STRAIN EDMONSTON	DQILRSMKGLSSTSI	1020	P69353.1
138	MEASLES MORBILLIVIRUS	DQYCADVAAEELMNA	1021	P06830.1
139	MEASLES VIRUS STRAIN EDMONSTON	DSESGGHITH	1022	P08362.1
140	MEASLES VIRUS STRAIN EDMONSTON	DTASESSQDPQDS	1023	P04851.1
141	RUBELLA VIRUS	DTVMSVFALASYVQH	1024	BAA28178.1
142	BORDETELLA PERTUSSIS	DVFQNGFTAWGNND	1025	P04977.1
143	RUBELLA VIRUS	DVGAVPPGKFVTAAL	1026	BAA28178.1
144	CORYNEBACTERIUM DIPHTHERIAE	DVNKSKTHISVNGRKI	1027	CAE11230.1
145	RUBELLA VIRUS	DVSCEGLGAWVPAAP	1028	BAA28178.1
146	RUBELLA VIRUS STRAIN THERIEN	DWASPVCQRHSPDCSRLVGATPERPRL	1029	P07566.1
147	MEASLES VIRUS STRAIN EDMONSTON	EARESYRETGPSR	1030	P04851.1
148	BORDETELLA PERTUSSIS	EAVEAERAGRGTG	1031	ACI04548.1
149	MEASLES VIRUS STRAIN EDMONSTON	EDAKELLESSDQILR	1032	P69353.1
150	MEASLES VIRUS STRAIN EDMONSTON	EDRRVKQSRGEAR	1033	P04851.1
151	MEASLES VIRUS STRAIN EDMONSTON	EDSITIPYQGSGKGV	1034	P08362.1
152	RUBELLA VIRUS	EEAFTYLCTAPGCAT	1035	BAA28178.1
153	MEASLES VIRUS STRAIN EDMONSTON	EGFNMILGTILAQIW	1036	P04851.1
154	MEASLES VIRUS STRAIN EDMONSTON	EGFNMILGTILAQIWVLLAK	1037	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
155	RUBELLA VIRUS	EHPFCNTPHGQLEVQ	1038	BAA28178.1
156	MEASLES VIRUS STRAIN EDMONSTON	EISDIEVQDPEGFNM	1039	P04851.1
157	MEASLES VIRUS STRAIN EDMONSTON	EISDIEVQDPEGFNMILGTI	1040	P04851.1
158	MEASLES VIRUS STRAIN EDMONSTON	EKPNLSSKRSE	1041	P08362.1
159	MEASLES VIRUS STRAIN EDMONSTON	ELKLAALCHGEDSIT	1042	P08362.1
160	MEASLES MORBILLIVIRUS	ELMNALVNSTLLETR	1043	P06830.1
161	MEASLES VIRUS STRAIN EDMONSTON	ELPRL	1044	P04851.1
162	MEASLES MORBILLIVIRUS	ENPEWAPLKDNRIPSYGVLSVDL	1045	AAR89413.1
163	MEASLES VIRUS STRAIN EDMONSTON	EPIRDALNAMTQNIR	1046	P69353.1
164	MEASLES VIRUS STRAIN EDMONSTON	EQVGMSRPGLKPDLT	1047	P69353.1
165	RUBELLA VIRUS	ERPRLRLV	1048	CAJ88851.1
166	RUBELLA VIRUS	ERPRLRLVDADDPLL	1049	BAA28178.1
167	MEASLES VIRUS CAM/RB	ESPGQLIQRITDDPDVS	1050	P04851.1
168	MEASLES VIRUS STRAIN EDMONSTON	ESRGIKARITHVDTE	1051	P69353.1
169	MEASLES VIRUS STRAIN EDMONSTON	ESSCTFMPEGTVCSQ	1052	P69353.1
170	MEASLES VIRUS STRAIN EDMONSTON	ESSQDPQDSRRSA	1053	P04851.1
171	MEASLES VIRUS STRAIN EDMONSTON	ETRTTNQFLAVSKGN	1054	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
172	MEASLES VIRUS STRAIN EDMONSTON	EVDGDVKLSSNLVIL	1055	P08362.1
173	MEASLES VIRUS STRAIN EDMONSTON-B	EVNGVTIQV	1056	P26031.1
174	RUBELLA VIRUS	EVWVTPVI	1057	CAJ88851.1
175	RUBELLA VIRUS	EVWVTPVIGSQA	1058	BAA19893.1
176	RUBELLA VIRUS	EWAAAHWWQLTLGAT	1059	BAA28178.1
177	MEASLES MORBILLIVIRUS	EWIPRFKVSPYLFTV	1060	P06830.1
178	BORDETELLA PERTUSSIS	FEYVDTYGDNAG	1061	P04977.1
179	MEASLES MORBILLIVIRUS	FGPLITHGSGMDLYK	1062	P06830.1
180	MEASLES VIRUS STRAIN EDMONSTON	FIFDALAEV	1063	ABK40531.1
181	MEASLES VIRUS STRAIN EDMONSTON	FISDKIKFL	1064	P08362.1
182	MEASLES VIRUS STRAIN EDMONSTON	FKRNKDKPPITSGSG	1065	P04851.1
183	MEASLES VIRUS STRAIN EDMONSTON	FKRNKDKPPITSGSGGAIRG	1066	P04851.1
184	RUBELLA VIRUS	FKTVRPVALPRTLAP	1067	BAA28178.1
185	MEASLES VIRUS STRAIN EDMONSTON	FLMDRHIIV	1068	ABK40531.1
186	MEASLES VIRUS STRAIN EDMONSTON	FMAVLLTLQTPTGQI	1069	P69353.1
187	MEASLES VIRUS STRAIN EDMONSTON	FMPEGTVCSQNALYP	1070	P69353.1
188	MEASLES MORBILLIVIRUS	FMYMSLLGV	1071	AAN09804.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
189	MEASLES VIRUS STRAIN EDMONSTON	FNVPIKEAGEDCHAP	1072	P08362.1
190	MEASLES MORBILLIVIRUS	FRDLTWCINPPERIK	1073	AAC35876.2
191	MEASLES VIRUS STRAIN EDMONSTON	FSHDDPISSDQSRFG	1074	P04851.1
192	MEASLES VIRUS STRAIN EDMONSTON	FSHDDPISSDQSRFGWFENK	1075	P04851.1
193	MEASLES MORBILLIVIRUS	FTDLVKFISDKIKFL	1076	P06830.1
194	MEASLES MORBILLIVIRUS	FTWDQKLWCRHFCVL	1077	P06830.1
195	BORDETELLA PERTUSSIS	FVRDGQSVIGACASPYEGRYRDLYDALRRLLY	1078	SRC280066
196	BORDETELLA PERTUSSIS	FVRSGQPVIGACTSPYDGKYWSILYSRLRKMLY	1079	SRC280066
197	MEASLES MORBILLIVIRUS	FYKDNPHPKGSRIVI	1080	P06830.1
198	BORDETELLA PERTUSSIS	GAASSYFEYVDTYG	1081	ACI04548.1
199	BORDETELLA PERTUSSIS	GAFDLKTTFCIMTTRNTGQPA	1082	AAA83981.1
200	MEASLES VIRUS STRAIN EDMONSTON	GALIGILSLFVESPG	1083	P04851.1
201	MEASLES VIRUS STRAIN EDMONSTON	GALIGILSLFVESPGQLIQR	1084	P04851.1
202	RUBELLA VIRUS	GATPERPR	1085	CAJ88851.1
203	BORDETELLA PERTUSSIS	GAYGRCPNGTRALTVAELRGNAEL	1086	P04979.1
204	RUBELLA VIRUS	GCFAPWDLEATGACI	1087	BAA28178.1
205	MEASLES MORBILLIVIRUS	GDINKVLEKLGYS	1088	BAB60865.1
206	MEASLES MORBILLIVIRUS	GDINKVLEKLGYSGGDLLG	1089	AAL29688.1
207	RUBELLA VIRUS	GDLRAVHHRPVPA	1090	CAA28880.1
208	RUBELLA VIRUS	GDLVEYIMNYTGNQQ	1091	BAA28178.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
209	MEASLES VIRUS STRAIN EDMONSTON	GDSSITTRSRLLDRL	1092	P04851.1
210	MEASLES VIRUS STRAIN EDMONSTON	GDSSITTRSRLLDRLVRLIG	1093	P04851.1
211	MEASLES MORBILLIVIRUS	GEDCHAPTYLPAEVD	1094	P06830.1
212	MEASLES VIRUS STRAIN EDMONSTON	GELSTLESLMNLYQQ	1095	P04851.1
213	MEASLES VIRUS STRAIN EDMONSTON	GELSTLESLMNLYQQMGKPA	1096	P04851.1
214	MUMPS RUBULAVIRUS	GEQARYLALLEA	1097	P21186.1
215	RUBELLA VIRUS	GEVWVT	1098	BAA19893.1
216	RUBELLA VIRUS	GEVWVTPV	1099	CAJ88851.1
217	RUBELLA VIRUS	GEVWVTPVIGSQAR	1100	BAA19893.1
218	BORDETELLA PERTUSSIS	GEYGGVIKDGTPGGA	1101	AAA83981.1
219	RUBELLA VIRUS	GFLSGVGPMRLRHGADT	1102	SRC265968
220	MEASLES VIRUS STRAIN EDMONSTON-B	GFRASDVETAEGGEIHELLRLQ	1103	P03422.1
221	BORDETELLA PERTUSSIS	GGAVPGGAVPGGAVPGGFGPGGFGP	1104	P14283.3
222	BORDETELLA PERTUSSIS	GGAVPGGAVPGGFGPGGFGPGGFGP	1105	CAA09475.1
223	BORDETELLA PERTUSSIS	GGAVPGGAVPGGFGPGGFGPGGFGP GGFGP	1106	CAA09474.1
224	MEASLES MORBILLIVIRUS	GGHITHSGMVGMGVS	1107	P06830.1
225	MEASLES MORBILLIVIRUS	GILESRGIKARITHVDTESY	1108	P26032.1
226	BORDETELLA PERTUSSIS	GITGETTTTEYSNARYV	1109	CAD44970.1
227	MEASLES VIRUS STRAIN EDMONSTON	GKEDRRVKQSRGE	1110	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
228	BORDETELLA PERTUSSIS	GKVTNGS	1111	ACI16088.1
229	RUBELLA VIRUS	GLGAWVPAAPCARIW	1112	BAA28178.1
230	MEASLES VIRUS STRAIN EDMONSTON	GLIGIPALICCCRGR	1113	P69353.1
231	RUBELLA VIRUS STRAIN THERIEN	GLLACCAKCLYYLRGAIAPR	1114	P07566.1
232	MEASLES MORBILLIVIRUS	GLLAIAGIRLHRAAI	1115	P06830.1
233	RUBELLA VIRUS	GLQPRADMAAPPTLPQ	1116	NP_740663.1
234	MEASLES MORBILLIVIRUS	GMGVSCTVTREDGTNRR	1117	AAR89413.1
235	MEASLES MORBILLIVIRUS	GMYGGTYLVEKP	1118	AAR89413.1
236	BORDETELLA PERTUSSIS	GNAELQTYLRQITPGWSIYGLYDGTYLG	1119	P04979.1
237	RUBELLA VIRUS	GNCHLTVNGEDVGAV	1120	BAA28178.1
238	BORDETELLA PERTUSSIS	GNNDNVLDHLTGR	1121	P04977.1
239	BORDETELLA PERTUSSIS	GNNDNVLDHLTGRSC	1122	P04977.1
240	MEASLES VIRUS STRAIN EDMONSTON	GNRFILSQGNLIANC	1123	P69353.1
241	RUBELLA VIRUS	GNRGRGQRRDWSRAPPPPEERQETRSQTPAPKPS	1124	P07566.1
242	RUBELLA VIRUS	GPGEVWVT	1125	CAJ88851.1
243	RUBELLA VIRUS STRAIN THERIEN	GPMRLRHGADTRCGRLI	1126	P07566.1
244	BORDETELLA PERTUSSIS	GPNHTKV	1127	ACI16083.1
245	MEASLES VIRUS STRAIN HALLE	GPRQAQVSF	1128	P10050.1
246	MEASLES VIRUS STRAIN EDMONSTON	GPRQAQVSFLQGDQS	1129	P04851.1
247	MEASLES VIRUS STRAIN EDMONSTON	GPRQAQVSFLQGDQSENELP	1130	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
248	MEASLES MORBILLIVIRUS	GRGYNVSSIVTMTSQ	1131	P06830.1
249	BORDETELLA PERTUSSIS	GRTPFII	1132	ACI16083.1
250	RUBELLA VIRUS	GSPNCHGPDWASPVC	1133	BAA28178.1
251	RUBELLA VIRUS	GSQARKCGLHIRAGP	1134	BAA28178.1
252	BORDETELLA PERTUSSIS	GSSNSAFVSTSSSRR	1135	P04977.1
253	MEASLES VIRUS STRAIN EDMONSTON	GSTKSCARTLVSGSF	1136	P69353.1
254	RUBELLA VIRUS	GSYYKQYHPTACEVE	1137	BAA28178.1
255	RUBELLA VIRUS	GTHTTAVSETRQTWA	1138	BAA28178.1
256	MEASLES VIRUS STRAIN EDMONSTON	GTIINQDPDKILTYI	1139	P69353.1
257	BORDETELLA PERTUSSIS	GTLVRMAPVIG	1140	ADA85124.1
258	MEASLES VIRUS STRAIN EDMONSTON	GTPLDIDTASESS	1141	P04851.1
259	BORDETELLA PERTUSSIS	GTYLGQAYGGIIKDAPPGAGFIYRETFC	1142	P04979.1
260	BORDETELLA PERTUSSIS	GVATKGLGVHAKSSDWG	1143	P15318.2
261	CORYNEBACTERIUM DIPHTHERIAE	GVLLPTIPGKLDVNKSKTHI	1144	AAV70486.1
262	MEASLES MORBILLIVIRUS	GVLSVDLSLTVELKI	1145	P06830.1
263	MEASLES MORBILLIVIRUS	GVPIELQVECFTWDQ	1146	P06830.1
264	MEASLES VIRUS STRAIN EDMONSTON	GVSCTVTREDGTNRR	1147	P08362.1
265	MEASLES VIRUS STRAIN EDMONSTON	GVSYNIGSQEWYTTV	1148	P69353.1
266	MEASLES VIRUS STRAIN EDMONSTON	GYNVSSIVTMTSQGM	1149	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
267	MEASLES MORBILLIVIRUS	HFCVLADSESGGHIT	1150	P06830.1
268	MEASLES MORBILLIVIRUS	HGEDSITIPYQGSGK	1151	P06830.1
269	RUBELLA VIRUS	HGPDWASP	1152	BAA19893.1
270	RUBELLA VIRUS	HGPDWASPVCQRHSP	1153	BAA28178.1
271	RUBELLA VIRUS	HGPDWASPVCQRHSPDCSRLVG	1154	CAJ88851.1
272	RUBELLA VIRUS STRAIN M33	HGPDWASPVCQRHSPDCSRLVGATPERPRLRLV	1155	CAJ88851.1
273	MEASLES VIRUS STRAIN EDMONSTON	HITHSGMEGMGVSCT	1156	P08362.1
274	MEASLES MORBILLIVIRUS	HKSLSTNLDVTNSIE	1157	P06830.1
275	MEASLES VIRUS STRAIN EDMONSTON	HLMIDRPYV	1158	P08362.1
276	MEASLES VIRUS STRAIN EDMONSTON	HLPTGTPLDIDTA	1159	P04851.1
277	MEASLES VIRUS STRAIN EDMONSTON-B	HLPTGTPLDIDTATESSQDPQDSR	1160	Q77M43.1
278	MEASLES MORBILLIVIRUS	HMTNYLEQPVSNDLS	1161	P06830.1
279	MEASLES VIRUS STRAIN EDMONSTON	HQSLVIKLMPNITLL	1162	P69353.1
280	MEASLES MORBILLIVIRUS	HRAAIYTAEIHKSLS	1163	P06830.1
281	BORDETELLA PERTUSSIS	HRMQEAVEAERAGRGTGH	1164	P04977.1
282	MEASLES VIRUS STRAIN EDMONSTON	HVDTESYFIVLSIAY	1165	P69353.1
283	MEASLES VIRUS STRAIN EDMONSTON	HWGNLSKIGVVGIGS	1166	P69353.1
284	RUBELLA VIRUS	HWWQLTLGATCALPL	1167	BAA28178.1
285	RUBELLA VIRUS	HYRNASDVLPGHWLQGGWGCYNL	1168	NP_740663.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
286	MEASLES VIRUS STRAIN EDMONSTON	IDLGPPISLERLDVG	1169	P69353.1
287	MEASLES VIRUS STRAIN EDMONSTON	IEAIRQAGQEMILAV	1170	P69353.1
288	RUBELLA VIRUS STRAIN M33	IETRSARHP	1171	CAA28880.1
289	MEASLES VIRUS STRAIN EDMONSTON	IGSQEWYTTVPKYVA	1172	P69353.1
290	MEASLES VIRUS STRAIN EDMONSTON	IKGVIVHRLEGVSYN	1173	P69353.1
291	MEASLES VIRUS STRAIN EDMONSTON	IKHIIIVPIPGDSSI	1174	P04851.1
292	MEASLES VIRUS STRAIN EDMONSTON	IKHIIIVPIPGDSSITTRSR	1175	P04851.1
293	BORDETELLA PERTUSSIS	IKLKDCP	1176	ACI16083.1
294	MEASLES VIRUS STRAIN EDMONSTON	IKLMPNITLLNNCTR	1177	P69353.1
295	MEASLES MORBILLIVIRUS	ILLERLDVGT	1178	AAF85664.1
296	MEASLES MORBILLIVIRUS	ILPGQDLQYV	1179	P08362.1
297	MEASLES VIRUS STRAIN EDMONSTON	ILTYIAADHCPVVEV	1180	P69353.1
298	MEASLES MORBILLIVIRUS	INQDPDKILTY	1181	AAL29688.1
299	MEASLES VIRUS STRAIN EDMONSTON	IPRFKVSPYLFNVPI	1182	P08362.1
300	MEASLES VIRUS STRAIN EDMONSTON	IQALSYALGGDINKV	1183	P69353.1
301	RUBELLA VIRUS	IRAGPYGHATVEMPE	1184	BAA28178.1
302	BORDETELLA PERTUSSIS	IRMGTDK	1185	ACI16088.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
303	MEASLES VIRUS STRAIN EDMONSTON	ISNFDESSCTFMPEG	1186	P69353.1
304	MEASLES VIRUS STRAIN EDMONSTON	ITAGIALHQSMLNSQ	1187	P69353.1
305	MEASLES VIRUS STRAIN EDMONSTON	ITDDPDVSIRLLEVV	1188	P04851.1
306	MEASLES VIRUS STRAIN EDMONSTON	ITDDPDVSIRLLEVVQSDQS	1189	P04851.1
307	BORDETELLA PERTUSSIS	ITTYV	1190	ACI16083.1
308	MEASLES VIRUS STRAIN EDMONSTON	IVEAGLASFILTIKF	1191	P04851.1
309	MEASLES VIRUS STRAIN EDMONSTON	IVEAGLASFILTIKFGIETM	1192	P04851.1
310	MEASLES VIRUS STRAIN EDMONSTON	IVEAGLASFILTIKFGIETMYPALG	1193	P04851.1
311	RUBELLA VIRUS	KALEAQSRALRAELAA	1194	P07566.1
312	MEASLES VIRUS STRAIN EDMONSTON	KARITHVDTESYFIV	1195	P69353.1
313	RUBELLA VIRUS	KCGLHIRAGPYGHAT	1196	BAA28178.1
314	MEASLES VIRUS STRAIN EDMONSTON	KCYTTGTIINQDPDK	1197	P69353.1
315	MEASLES VIRUS STRAIN EDMONSTON	KDNPHPKGSR	1198	P08362.1
316	MEASLES VIRUS STRAIN EDMONSTON	KDNRIPSYGVLSVDL	1199	P08362.1
317	MEASLES MORBILLIVIRUS	KFLNPDREYDFRDLT	1200	AAC35876.2
318	RUBELLA VIRUS	KFVTAALLN	1201	BAA28178.1
319	MEASLES VIRUS STRAIN EDMONSTON	KGNCSGPTTIR	1202	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
320	MEASLES MORBILLIVIRUS	KIKFLNPDREYDFRD	1203	P06830.1
321	RUBELLA VIRUS	KIVDGGCFAPWDLEA	1204	BAA28178.1
322	MEASLES VIRUS STRAIN EDMONSTON	KLGLKLLRYYTEILS	1205	P69353.1
323	MEASLES VIRUS STRAIN EDMONSTON	KLGVWKSPTDMQSWV	1206	P08362.1
324	BORDETELLA PERTUSSIS	KLKECPQ	1207	ACI16088.1
325	MEASLES MORBILLIVIRUS	KLLRYYTEI	1208	P26031.1
326	MEASLES VIRUS STRAIN EDMONSTON	KLMPFSGDFV	1209	ABK40531.1
327	MEASLES MORBILLIVIRUS	KLMPNITLL	1210	P26031.1
328	MEASLES MORBILLIVIRUS	KLRMETCFQQACKGKIQALCENPEWA	1211	AAR89413.1
329	MEASLES MORBILLIVIRUS	KLWCRHFCV	1212	P08362.1
330	MEASLES VIRUS STRAIN EDMONSTON	KLWCRHFCVL	1213	P08362.1
331	MEASLES MORBILLIVIRUS	KLWESPQEI	1214	BAB60863.1
332	MEASLES VIRUS STRAIN EDMONSTON	KMSSAVGFV	1215	ABO69699.1
333	MEASLES VIRUS STRAIN EDMONSTON-B	KMSSAVGFVPDTGPASR	1216	P03422.1
334	BORDETELLA PERTUSSIS	KMVYATN	1217	ACI16083.1
335	MEASLES VIRUS STRAIN EDMONSTON	KPDLTGTSKSYVRSL	1218	P69353.1
336	MEASLES MORBILLIVIRUS	KPNLSSKRSELSQLS	1219	P08362.1
337	MEASLES VIRUS STRAIN EDMONSTON	KPNLSSKRSELSQLSMYRVF	1220	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
338	MEASLES VIRUS STRAIN EDMONSTON	KQSRGEARESYRETG	1221	P04851.1
339	MEASLES VIRUS STRAIN EDMONSTON	KQSRGEARESYRETGPSRAS	1222	P04851.1
340	MEASLES VIRUS STRAIN EDMONSTON	KRFAGVVLAGAALGV	1223	P69353.1
341	MEASLES VIRUS STRAIN EDMONSTON	KRTPGNKPRIAEMIC	1224	P04851.1
342	MEASLES VIRUS STRAIN EDMONSTON	KRTPGNKPRIAEMICDIDTY	1225	P04851.1
343	MEASLES MORBILLIVIRUS	KSNHNNVYWLTIPPMKNLALGVINTL	1226	AAR89413.1
344	MEASLES MORBILLIVIRUS	KVSPYLFNV	1227	P08362.1
345	BORDETELLA PERTUSSIS	KVVQLPKISKNALKANG	1228	ACI16083.1
346	BORDETELLA PERTUSSIS	KVVQLPKISKNALRNDG	1229	ACI16087.1
347	BORDETELLA PERTUSSIS	LAHRRIPPENIR	1230	P04977.1
348	BORDETELLA PERTUSSIS	LALALWAGFALS	1231	P11092.1
349	RUBELLA VIRUS	LAPGGGNCHLTVNGE	1232	BAA28178.1
350	MEASLES VIRUS STRAIN EDMONSTON	LAQIWVLLAKAVTAP	1233	P04851.1
351	MEASLES VIRUS STRAIN EDMONSTON	LAQIWVLLAKAVTAPDTAAD	1234	P04851.1
352	RUBELLA VIRUS	LASYFNPGGSYYKQYHPTACEVEPAFGHS	1235	BAA19893.1
353	MEASLES MORBILLIVIRUS	LAVSKGNCSGPTTIR	1236	P06830.1
354	MEASLES VIRUS STRAIN EDMONSTON	LCENPEWAPLKDNRI	1237	P08362.1
355	MEASLES MORBILLIVIRUS	LDRLVRLIG	1238	ABI54110.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
356	CORYNEBACTERIUM DIPHTHERIAE	LEEEGVTPL	1239	P33120.2
357	BORDETELLA PERTUSSIS	LEHRMQEAVEAERAGRGTGHFI	1240	CAD44970.1
358	MEASLES VIRUS STRAIN EDMONSTON	LEKLGYSGGDLLGIL	1241	P69353.1
359	MEASLES VIRUS STRAIN EDMONSTON	LEQPVSNDLS	1242	P08362.1
360	MEASLES VIRUS STRAIN EDMONSTON	LERKWLDVVRNIIAE	1243	P04851.1
361	MEASLES VIRUS STRAIN EDMONSTON	LERKWLDVVRNIIAEDLSLR	1244	P04851.1
362	MEASLES VIRUS STRAIN EDMONSTON	LFGPSLRDPISAEIS	1245	P69353.1
363	MEASLES MORBILLIVIRUS	LGELKLAALCHGEDS	1246	P06830.1
364	MEASLES VIRUS STRAIN EDMONSTON	LGGKEDRRVKQSR	1247	P04851.1
365	RUBELLA VIRUS	LGHDGHHGGTLRVGQHHRNASDVL	1248	ABD64214.1
366	RUBELLA VIRUS STRAIN THERIEN	LGSPNCHGPDWASPVCQRHS	1249	P07566.1
367	RUBELLA VIRUS STRAIN THERIEN	LGSPNCHGPDWASPVCQRHSPDCSRLV	1250	P07566.1
368	RUBELLA VIRUS	LHDPDTEAPTEACVTSWL	1251	ABD64214.1
369	MEASLES VIRUS STRAIN EDMONSTON	LIANCASILCKCYTT	1252	P69353.1
370	MEASLES MORBILLIVIRUS	LIGLLAIAGIRLHRAAIYTAEIHK	1253	AAR89413.1
371	MEASLES VIRUS STRAIN EDMONSTON	LIPSMNQLSCDLIGQ	1254	P69353.1
372	MEASLES VIRUS STRAIN EDMONSTON	LKIKIASGFGPLITH	1255	P08362.1
373	MEASLES MORBILLIVIRUS	LKIKIASGFGPLITHGSGMDLYK	1256	AAR89413.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
374	BORDETELLA PERTUSSIS	LKLYFEP	1257	ACI16088.1
375	MEASLES VIRUS STRAIN EDMONSTON	LLAVLFVMFL	1258	P08362.1
376	MEASLES VIRUS STRAIN EDMONSTON	LLDRLVRLIGNPDVS	1259	P04851.1
377	MEASLES VIRUS STRAIN EDMONSTON	LLDRLVRLIGNPDVSGPKLT	1260	P04851.1
378	MEASLES VIRUS STRAIN EDMONSTON	LLESSDQILRSMKGL	1261	P69353.1
379	MEASLES MORBILLIVIRUS	LLETRTTNQFLAVSK	1262	P06830.1
380	MEASLES VIRUS STRAIN EDMONSTON	LLEVVQSDQSQSGLT	1263	P04851.1
381	MEASLES VIRUS STRAIN EDMONSTON	LLEVVQSDQSQSGLTFASR	1264	P04851.1
382	MEASLES VIRUS STRAIN EDMONSTON	LLEVVQSDQSQSGLTFASRG	1265	P04851.1
383	MEASLES MORBILLIVIRUS	LLGILESRGIKARIT	1266	AAL29688.1
384	RUBELLA VIRUS	LLRTAPGP	1267	CAJ88851.1
385	MEASLES VIRUS STRAIN EDMONSTON	LLRYYTEILSLFGPS	1268	P69353.1
386	RUBELLA VIRUS STRAIN THERIEN	LLVPWVLIFMVCRRACRRRG	1269	P07566.1
387	MEASLES VIRUS STRAIN EDMONSTON	LLWRSRCKIV	1270	ABK40528.1
388	MEASLES MORBILLIVIRUS	LLWSYAMGV	1271	P04851.1
389	MEASLES VIRUS STRAIN EDMONSTON	LLWSYAMGVGVELEN	1272	P04851.1
390	MEASLES VIRUS STRAIN EDMONSTON	LLWSYAMGVGVELENSMGGL	1273	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
391	MEASLES MORBILLIVIRUS	LMIDRPYVL	1274	P08362.1
392	MEASLES VIRUS STRAIN EDMONSTON	LNAMTQNIRPVQSVA	1275	P69353.1
393	RUBELLA VIRUS	LNTPPPYQVSCGGES	1276	BAA28178.1
394	MEASLES VIRUS STRAIN EDMONSTON	LRDPISAEISIQALS	1277	P69353.1
395	BORDETELLA PERTUSSIS	LRGSGDLQEYLRHVTR	1278	AAA83981.1
396	RUBELLA VIRUS	LRLVDADD	1279	CAJ88851.1
397	RUBELLA VIRUS	LRLVDADDPLLR	1280	BAA19893.1
398	RUBELLA VIRUS	LRLVDADDPLLRTAPGPGEVWVTPVIGSQAR	1281	BAA19893.1
399	BORDETELLA PERTUSSIS	LRRLLYMIYMSGLAVRVHVSKEEQYYDY	1282	P04979.1
400	RUBELLA VIRUS	LRTAPGPG	1283	CAJ88851.1
401	RUBELLA VIRUS	LRVGQHYRNASDVLPGHWLQ	1284	NP_740663.1
402	BORDETELLA PERTUSSIS	LRYLA	1285	ACI16088.1
403	MEASLES VIRUS STRAIN EDMONSTON	LSCKPWQESRKNK	1286	P04851.1
404	MEASLES MORBILLIVIRUS	LSEIKGVIVHRLEGV	1287	AAL29688.1
405	MEASLES VIRUS STRAIN EDMONSTON	LSIAYPTLSEIKGVI	1288	P69353.1
406	MEASLES VIRUS STRAIN EDMONSTON	LSLLDLYLGRGYNVS	1289	P08362.1
407	MEASLES VIRUS STRAIN EDMONSTON	LSQGNLIANCASILC	1290	P69353.1
408	MEASLES MORBILLIVIRUS	LSSHRGVIADNQAKW	1291	P06830.1
409	MEASLES VIRUS STRAIN EDMONSTON	LSVDLSLTVELKIKI	1292	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
410	BORDETELLA PERTUSSIS	LTGISICNPGSSLC	1293	AAA83981.1
411	MEASLES VIRUS STRAIN EDMONSTON	LTIKFGIETMYPALG	1294	P04851.1
412	MEASLES VIRUS STRAIN EDMONSTON	LTIKFGIETMYPALGLHEFA	1295	P04851.1
413	MEASLES VIRUS STRAIN EDMONSTON	LTLQTPTGQIHWGNL	1296	P69353.1
414	RUBELLA VIRUS	LVDADDPL	1297	CAJ88851.1
415	RUBELLA VIRUS	LVDADDPLLR	1298	BAA19893.1
416	MEASLES VIRUS STRAIN EDMONSTON	LVEKPNLSSKRSELS	1299	P08362.1
417	RUBELLA VIRUS	LVGATPE	1300	BAA19893.1
418	RUBELLA VIRUS	LVGATPER	1301	CAJ88851.1
419	MEASLES MORBILLIVIRUS	LVKLGVWKSPTGMQS	1302	P06830.1
420	MEASLES VIRUS STRAIN EDMONSTON-B	LVSGSFGNRFILSQGNLI	1303	P26031.1
421	MEASLES VIRUS STRAIN EDMONSTON	LYKSNHNNVYWLTIP	1304	P08362.1
422	MEASLES VIRUS STRAIN EDMONSTON	LYPMSPLLQECLRGSTKSCARTLVS	1305	P69353.1
423	RUBELLA VIRUS	MASTTPITMEDLQKALEA	1306	P07566.1
424	RUBELLA VIRUS	MASTTPITMEDLQKALEAQSR	1307	ABD64200.1
425	RUBELLA VIRUS STRAIN THERIEN	MASTTPITMEDLQKALEAQSRALRAELAA	1308	P07566.1
426	RUBELLA VIRUS	MASTTPITMEDLQKALEAQSRALRAGLAA	1309	ABD64200.1
427	RUBELLA VIRUS VACCINE STRAIN RA27/3	MASTTPITMEDLQKALETQSRVLRAGLTA	1310	CAA33016.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
428	MEASLES VIRUS STRAIN EDMONSTON	MATLLRSLALFKRNK	1311	P04851.1
429	MEASLES VIRUS STRAIN EDMONSTON	MATLLRSLALFKRNKDKPPI	1312	P04851.1
430	MEASLES MORBILLIVIRUS	MDLYKSNHNNVYWLT	1313	P06830.1
431	RUBELLA VIRUS	MEDLQKALEAQSRA	1314	P07566.1
432	RUBELLA VIRUS	MEDLQKALEAQSRALRAELAA	1315	P07566.1
433	MEASLES VIRUS STRAIN EDMONSTON	MGLKVNVSAIFMAVL	1316	P69353.1
434	MEASLES MORBILLIVIRUS	MIDRPYVLLAVLFVM	1317	P06830.1
435	MEASLES VIRUS STRAIN EDMONSTON	MILAVQGVQDYINNE	1318	P69353.1
436	MEASLES VIRUS STRAIN EDMONSTON	MLNSQAIDNLRASLE	1319	P69353.1
437	MEASLES VIRUS STRAIN EDMONSTON	MNALVNSTLLETRTT	1320	P08362.1
438	RUBELLA VIRUS	MNYTGNQQSRWGLGSPNCHGPDWASPVCQRHS	1321	BAA19893.1
439	MEASLES VIRUS STRAIN EDMONSTON	MQSWVPLSTDDPVID	1322	P08362.1
440	MEASLES MORBILLIVIRUS	MSLSLLDLYLGRGYN	1323	P06830.1
441	MEASLES VIRUS STRAIN EDMONSTON	MSPLLQECLRGSTKS	1324	P69353.1
442	MEASLES MORBILLIVIRUS	MSPQRDRINAFYKDN	1325	P06830.1
443	MEASLES MORBILLIVIRUS	MYRVFEVSVIRNPGL	1326	P06830.1
444	MEASLES VIRUS STRAIN EDMONSTON	NALYPMSPLLQECLR	1327	P69353.1
445	RUBELLA VIRUS STRAIN THERIEN	NCHGPDWASPVCQRHSPDCSRLVGAT	1328	P07566.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
446	MEASLES VIRUS STRAIN EDMONSTON	NFGRSYFDPAYFRLG	1329	P04851.1
447	MEASLES VIRUS STRAIN EDMONSTON	NFGRSYFDPAYFRLGQEMVR	1330	P04851.1
448	RUBELLA VIRUS	NGTQRACTFWAVNAY	1331	BAA28178.1
449	MEASLES VIRUS STRAIN EDMONSTON	NGVTIQVGSRRYPDA	1332	P69353.1
450	MEASLES VIRUS STRAIN EDMONSTON	NIIAEDLSLRRFMVA	1333	P04851.1
451	MEASLES VIRUS STRAIN EDMONSTON	NIIAEDLSLRRFMVALILDI	1334	P04851.1
452	MEASLES VIRUS STRAIN EDMONSTON	NITLLNNCTRVEIAE	1335	P69353.1
453	MEASLES MORBILLIVIRUS	NLALGVINTLEWIPR	1336	P06830.1
454	CORYNEBACTERIUM DIPHTHERIAE	NLFQVVHWSYNRPAYSPG	1337	SRC280292
455	MEASLES VIRUS STRAIN EDMONSTON	NLVILPGQDLQYVLA	1338	P08362.1
456	MEASLES VIRUS STRAIN EDMONSTON	NLYQQMGKPAPYMVN	1339	P04851.1
457	MEASLES VIRUS STRAIN EDMONSTON	NLYQQMGKPAPYMVNLENSI	1340	P04851.1
458	MEASLES VIRUS STRAIN EDMONSTON	NNCTRVEIAEYRRLL	1341	P69353.1
459	MEASLES VIRUS STRAIN EDMONSTON	NPDREYDFRD	1342	P08362.1
460	MEASLES VIRUS STRAIN HALLE	NPDVSGPKL	1343	P10050.1
461	MEASLES VIRUS STRAIN EDMONSTON	NPDVSGPKLTGALIG	1344	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
462	MEASLES VIRUS STRAIN EDMONSTON	NPDVSGPKLTGALIGILSLF	1345	P04851.1
463	MEASLES MORBILLIVIRUS	NPPERIKLDYDQYCA	1346	P06830.1
464	MEASLES MORBILLIVIRUS	NQAKWAVPTTRTDDK	1347	P06830.1
465	MEASLES MORBILLIVIRUS	NQDPDKILTYIAADH	1348	AAF02706.1
466	MEASLES VIRUS STRAIN EDMONSTON	NQLSCDLIGQKLGLK	1349	P69353.1
467	RUBELLA VIRUS	NQQSRWGLGSPNCHGPDWASPVCQRHS	1350	ABD64214.1
468	MUMPS RUBULAVIRUS	NSTLGVKSAREF	1351	ABP48111.1
469	RUBELLA VIRUS	NTPHGQLEVQVPPDP	1352	BAA28178.1
470	MEASLES VIRUS STRAIN EDMONSTON	NVSAIFMAVLLTLQT	1353	P69353.1
471	MEASLES MORBILLIVIRUS	PAEVDGDVKLSSNLV	1354	P06830.1
472	RUBELLA VIRUS	PAFGHSDAACWGFPT	1355	BAA28178.1
473	MEASLES VIRUS STRAIN EDMONSTON	PALICCCRGRCNKKG	1356	P69353.1
474	MEASLES MORBILLIVIRUS	PDKILTYIAADHC	1357	AAF02706.1
475	MEASLES VIRUS STRAIN EDMONSTON	PERIKLDYDQYCADV	1358	P08362.1
476	RUBELLA VIRUS	PERPRLRL	1359	CAJ88851.1
477	RUBELLA VIRUS	PGCATQAPVPVRLAG	1360	BAA28178.1
478	RUBELLA VIRUS VACCINE STRAIN RA27/3	PGCATQAPVPVRLAGVRFESKIVDGGCFA	1361	CAJ88851.1
479	RUBELLA VIRUS	PGEVWVTP	1362	CAJ88851.1
480	RUBELLA VIRUS	PGEVWVTPVIGSQAR	1363	BAA28178.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
481	CORYNEBACTERIUM DIPHTHERIAE	PGKLDVNKSKTHISVN	1364	CAE11230.1
482	MEASLES VIRUS STRAIN EDMONSTON	PGLGAPVFHMTNYLE	1365	P08362.1
483	RUBELLA VIRUS	PGPGEVWV	1366	CAJ88851.1
484	RUBELLA VIRUS	PHKTVRVKFHTETRT	1367	BAA28178.1
485	MEASLES MORBILLIVIRUS	PIELQVECFTWDQKL	1368	AAR89413.1
486	MEASLES VIRUS STRAIN EDMONSTON-B	PISLERLDVG	1369	P26031.1
487	MEASLES VIRUS STRAIN EDMONSTON	PISLERLDVGTNLGN	1370	P69353.1
488	BORDETELLA PERTUSSIS	PKALFTQQGGAYGRC	1371	P04979.1
489	MEASLES VIRUS STRAIN EDMONSTON	PKYVATQGYLISNFD	1372	P69353.1
490	MEASLES VIRUS STRAIN EDMONSTON	PLDIDTASESSQD	1373	P04851.1
491	RUBELLA VIRUS	PLGLKFKTVRPVALP	1374	BAA28178.1
492	MEASLES VIRUS STRAIN EDMONSTON	PLITHGSGMDLYKSN	1375	P08362.1
493	MEASLES MORBILLIVIRUS	PLKDNRIPSYGVLSV	1376	P06830.1
494	RUBELLA VIRUS	PLLRTAPG	1377	CAJ88851.1
495	MEASLES VIRUS STRAIN EDMONSTON	PLLSCKPWQESRK	1378	P04851.1
496	BORDETELLA PERTUSSIS	PPATVYRYDSRPPE	1379	P04977.1
497	MEASLES MORBILLIVIRUS	PPISLERLDVGT	1380	AAL29688.1
498	RUBELLA VIRUS	PPPPEERQETRSQTPAPKPS	1381	P07566.1
499	BORDETELLA PERTUSSIS	PQEQITQHGSPYGRC	1382	AAA83981.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
500	BORDETELLA PERTUSSIS	PQEQITQHGSPYGRCANK	1383	AAA83981.1
501	BORDETELLA PERTUSSIS	PQPGPQPPQPPQPQPEAPAPQPPAG	1384	P14283.3
502	MEASLES VIRUS STRAIN EDMONSTON	PRLGGKEDRRVKQ	1385	P04851.1
503	RUBELLA VIRUS	PRLRLVDA	1386	CAJ88851.1
504	RUBELLA VIRUS	PRNVRVTGCYQCGTP	1387	BAA28178.1
505	MEASLES VIRUS STRAIN EDMONSTON	PSRASDARAAHLP	1388	P04851.1
506	MEASLES VIRUS STRAIN EDMONSTON	PTGQIHWGNLSKIGV	1389	P69353.1
507	MEASLES VIRUS STRAIN EDMONSTON	PTGTPLDIDTASE	1390	P04851.1
508	MEASLES VIRUS STRAIN EDMONSTON	PTLSEIKGVIVHRLE	1391	P69353.1
509	MEASLES MORBILLIVIRUS	PTTIRGQFSNMSLSL	1392	P06830.1
510	MEASLES MORBILLIVIRUS	PTTRTDDKLR	1393	AAR89413.1
511	MEASLES MORBILLIVIRUS	PTTRTDDKLRMETCFQQACKG	1394	AAR89413.1
512	RUBELLA VIRUS	PVALPRTLAPPRNVR	1395	BAA28178.1
513	CORYNEBACTERIUM DIPHTHERIAE	PVFAGANYAAWAVNVAQVI	1396	AAV70486.1
514	RUBELLA VIRUS	PVIGSQAR	1397	CAJ88851.1
515	MEASLES MORBILLIVIRUS	PVVEVNGVTIQVGSR	1398	AAL29688.1
516	RUBELLA VIRUS STRAIN THERIEN	PWELVVLTARPEDGWTCRGV	1399	P07566.1
517	MEASLES VIRUS STRAIN EDMONSTON	PWQESRKNKAQTR	1400	P04851.1
518	MEASLES VIRUS STRAIN EDMONSTON	PYMVNLENSIQNKFS	1401	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
519	MEASLES VIRUS STRAIN EDMONSTON	PYMVNLENSIQNKFSAGSYP	1402	P04851.1
520	MEASLES VIRUS STRAIN EDMONSTON	PYQGSGKGVS	1403	P08362.1
521	RUBELLA VIRUS	PYQVSCGGESDRASA	1404	BAA28178.1
522	MEASLES MORBILLIVIRUS	QACKGKIQALCEN	1405	P08362.1
523	MEASLES VIRUS STRAIN EDMONSTON	QAGQEMILAVQGVQD	1406	P69353.1
524	MEASLES MORBILLIVIRUS	QALCENPECVPLKDN	1407	P06830.1
525	BORDETELLA PERTUSSIS	QALGALK	1408	ACI16088.1
526	RUBELLA VIRUS	QAPVPVRLAGVRFES	1409	BAA28178.1
527	RUBELLA VIRUS	QCGTPALVEGLAPGG	1410	BAA28178.1
528	MEASLES VIRUS STRAIN EDMONSTON	QDPDKILTYIAADHC	1411	P69353.1
529	MEASLES VIRUS STRAIN EDMONSTON	QECLRGSTKSCARTL	1412	P69353.1
530	BORDETELLA PERTUSSIS	QEQITQHGSPYGRC	1413	AAA83981.1
531	MEASLES VIRUS STRAIN EDMONSTON	QESRKNKAQTRTP	1414	P04851.1
532	MEASLES VIRUS STRAIN EDMONSTON	QGDQSENELPRLGGK	1415	P04851.1
533	MEASLES VIRUS STRAIN EDMONSTON	QGDQSENELPRLGGKEDRRV	1416	P04851.1
534	CORYNEBACTERIUM DIPHTHERIAE	QGESGHDIKITAENTPLPIA	1417	AAV70486.1
535	MEASLES MORBILLIVIRUS	QGSGKGVSFQLVKLG	1418	P06830.1
536	MEASLES VIRUS STRAIN EDMONSTON	QGVQDYINNELIPSM	1419	P69353.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
537	RUBELLA VIRUS	QLEVQVPPDPGDLVE	1420	BAA28178.1
538	MEASLES MORBILLIVIRUS	QLPEATFMV	1421	ABK40528.1
539	RUBELLA VIRUS	QLPFLGHDGHHGGTLRVGQHYRNAS	1422	NP_740663.1
540	MEASLES VIRUS STRAIN EDMONSTON	QLSMYRVFEV	1423	P08362.1
541	BORDETELLA PERTUSSIS	QLSNIT	1424	ACI16083.1
542	MEASLES VIRUS STRAIN EDMONSTON	QNIRPVQSVASSRRH	1425	P69353.1
543	MEASLES VIRUS STRAIN EDMONSTON	QNKFSAGSYPLLWSY	1426	P04851.1
544	MEASLES VIRUS STRAIN EDMONSTON	QNKFSAGSYPLLWSYAMGVG	1427	P04851.1
545	MEASLES VIRUS STRAIN EDMONSTON	QNKFSAGSYPLLWSYAMGVGVELEN	1428	P04851.1
546	MEASLES VIRUS STRAIN EDMONSTON	QQACKGKIQALCENP	1429	P08362.1
547	MEASLES VIRUS STRAIN EDMONSTON	QQRRVVGEFRLERKW	1430	P04851.1
548	MEASLES VIRUS STRAIN EDMONSTON	QQRRVVGEFRLERKWLDVVR	1431	P04851.1
549	BORDETELLA PERTUSSIS	QQTRANPNPYTSRRSVAS	1432	P04977.1
550	RUBELLA VIRUS	QRHSPDCSRLVGATP	1433	BAA28178.1
551	MEASLES VIRUS STRAIN EDMONSTON	QSGLTFASRGTNMED	1434	P04851.1
552	MEASLES VIRUS STRAIN EDMONSTON	QSGLTFASRGTNMEDEADQY	1435	P04851.1
553	CORYNEBACTERIUM DIPHTHERIAE	QSIALSSLMVAQAIPLVGEL	1436	AAV70486.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
554	MEASLES VIRUS STRAIN EDMONSTON	QSRFGWFENKEISDI	1437	P04851.1
555	MEASLES VIRUS STRAIN EDMONSTON	QSRFGWFENKEISDIEVQDP	1438	P04851.1
556	MEASLES VIRUS STRAIN EDMONSTON	QSRGEAR	1439	P04851.1
557	MEASLES VIRUS STRAIN EDMONSTON	QSRGEARESYRETGPSRA	1440	P04851.1
558	RUBELLA VIRUS STRAIN THERIEN	QTGRGGSAPRPELGPPTN	1441	P07566.1
559	RUBELLA VIRUS STRAIN THERIEN	QTPAPKPSRAPPQQPQPPRMQTGRG	1442	P07566.1
560	MEASLES VIRUS STRAIN EDMONSTON	QVGSRRYPDAVYLHR	1443	P69353.1
561	MEASLES VIRUS STRAIN EDMONSTON	QVSFLQGDQSENE	1444	P04851.1
562	RUBELLA VIRUS	QYHPTACEVEPAFGH	1445	BAA28178.1
563	MEASLES VIRUS STRAIN EDMONSTON	QYVLATYDTSRVEHA	1446	P08362.1
564	BORDETELLA PERTUSSIS	RANPNPYTSRRSV	1447	ACI04548.1
565	MEASLES VIRUS STRAIN EDMONSTON	RASDARAAHLPTG	1448	P04851.1
566	MEASLES VIRUS STRAIN EDMONSTON	RASLETTNQAIEAIR	1449	P69353.1
567	RUBELLA VIRUS STRAIN THERIEN	RCGRLICGLSTTAQYPPTRF	1450	P07566.1
568	BORDETELLA PERTUSSIS	RDGQSVIGACASPYEGRYR	1451	P04979.1
569	MEASLES VIRUS STRAIN EDMONSTON	RESYRETGPSRAS	1452	P04851.1
570	MEASLES VIRUS STRAIN EDMONSTON	RETGPSRASDARA	1453	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
571	MUMPS RUBULAVIRUS	RFAKYQQQGRLEAR	1454	P21186.1
572	RUBELLA VIRUS STRAIN THERIEN	RFGAPQAFLAGLLLATVAVGTARA	1455	P07566.1
573	MEASLES VIRUS STRAIN EDMONSTON	RFMVALILDIKRTPG	1456	P04851.1
574	MEASLES VIRUS STRAIN EDMONSTON	RFMVALILDIKRTPGNKPRI	1457	P04851.1
575	MEASLES VIRUS STRAIN EDMONSTON	RGEARESYRETGP	1458	P04851.1
576	RUBELLA VIRUS	RGTTPPAYG	1459	CAA28880.1
577	RUBELLA VIRUS STRAIN M33	RIETRSARH	1460	ABD64214.1
578	BORDETELLA PERTUSSIS	RILAGALATYQ	1461	P04977.1
579	BORDETELLA PERTUSSIS	RIPPENIRRVT	1462	ACI04548.1
580	BORDETELLA PERTUSSIS	RISNLND	1463	ACI16083.1
581	BORDETELLA PERTUSSIS	RLANLNG	1464	ACI16088.1
582	MEASLES VIRUS STRAIN EDMONSTON	RLDVGTNLGNAIAKL	1465	P69353.1
583	MEASLES VIRUS STRAIN EDMONSTON	RLERKWLDV	1466	P04851.1
584	MEASLES VIRUS STRAIN EDMONSTON	RLGGKEDRRVKQSRG	1467	P04851.1
585	MEASLES VIRUS STRAIN EDMONSTON	RLGGKEDRRVKQSRGEARES	1468	P04851.1
586	MEASLES VIRUS STRAIN EDMONSTON	RLLDRLVRL	1469	ABI54110.1
587	RUBELLA VIRUS	RLRLVDAD	1470	CAJ88851.1
588	RUBELLA VIRUS	RLRLVDADDPLLR	1471	BAA19893.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
589	RUBELLA VIRUS	RLRLVDADDPLLRTAPGPGEVWVTPVIGSQA	1472	BAA19893.1
590	RUBELLA VIRUS	RLRLVQDADDPLLRIAPGPGEVWVTPVIGSQA	1473	SRC265968
591	MEASLES VIRUS STRAIN EDMONSTON	RLSDNGYYTV	1474	ABK40528.1
592	RUBELLA VIRUS	RLVDADDP	1475	CAJ88851.1
593	RUBELLA VIRUS	RLVDADDPLLRTAPG	1476	BAA28178.1
594	RUBELLA VIRUS	RLVGATPE	1477	CAJ88851.1
595	RUBELLA VIRUS	RMQTGRGGSAPRPELGPPTNPFQAAVA	1478	ABD64216.1
596	MEASLES MORBILLIVIRUS	RMSKGVFKV	1479	ABY21184.1
597	MEASLES MORBILLIVIRUS	RNPGLGAPVFHMTNY	1480	P06830.1
598	RUBELLA VIRUS	RPRLRLVD	1481	CAJ88851.1
599	BORDETELLA PERTUSSIS	RQAESSEAMAAWSERAGEA	1482	P04977.1
600	RUBELLA VIRUS	RQTWAEWAAAHWWQL	1483	BAA28178.1
601	MEASLES VIRUS STRAIN EDMONSTON	RRVKQSRGEARES	1484	P04851.1
602	MEASLES MORBILLIVIRUS	RRYPDAVYL	1485	ACA09725.1
603	MEASLES VIRUS STRAIN EDMONSTON	RRYPDAVYLHRIDLG	1486	P69353.1
604	MEASLES VIRUS STRAIN EDMONSTON	RSAGKVSSTLASELG	1487	P04851.1
605	MEASLES VIRUS STRAIN EDMONSTON	RSAGKVSSTLASELGITAED	1488	P04851.1
606	MEASLES VIRUS STRAIN EDMONSTON	RSELSQLS	1489	P08362.1
607	MEASLES VIRUS STRAIN EDMONSTON	RSELSQLSMYRVFEV	1490	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
608	RUBELLA VIRUS	RSQTPAPKPSRAPPQQPQPPRMQT	1491	ABD64214.1
609	RUBELLA VIRUS	RTAPGPGE	1492	CAJ88851.1
610	RUBELLA VIRUS	RTAPGPGEVWVTPVI	1493	BAA28178.1
611	MEASLES MORBILLIVIRUS	RTDDKLRMETCFQQA	1494	P06830.1
612	RUBELLA VIRUS	RTLAPPRNVRVTGCY	1495	BAA28178.1
613	MEASLES VIRUS STRAIN EDMONSTON	RTVLEPIRDALNAMT	1496	P69353.1
614	MEASLES VIRUS STRAIN EDMONSTON	RVEHAVVYYVYSPSR	1497	P08362.1
615	MEASLES VIRUS STRAIN EDMONSTON	RVFEVGVIRNPGLGA	1498	P08362.1
616	BORDETELLA PERTUSSIS	RVHVSKEEQYYDYEDATFE	1499	P04978.2
617	RUBELLA VIRUS	RVIDPAAQ	1500	BAA28178.1
618	RUBELLA VIRUS	RVKFHTETRTVWQLS	1501	BAA28178.1
619	BORDETELLA PERTUSSIS	RVYHNGITGET	1502	ACI04548.1
620	BORDETELLA PERTUSSIS	RYDSRPPEDVF	1503	ACI04548.1
621	MEASLES VIRUS STRAIN EDMONSTON	RYPDAVYLHRIDLGP	1504	P69353.1
622	MEASLES VIRUS STRAIN EDMONSTON	SAEISIQALSYALGG	1505	P69353.1
623	MEASLES VIRUS STRAIN EDMONSTON	SAEPLLSCKPWQESR	1506	P04851.1
624	MEASLES VIRUS STRAIN EDMONSTON	SAEPLLSCKPWQESRKNKAQ	1507	P04851.1
625	MEASLES MORBILLIVIRUS	SAGKVSSTLASELG	1508	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
626	MEASLES VIRUS STRAIN EDMONSTON	SAGKVSSTLASELGITAEDARLVS	1509	P04851.1
627	MEASLES VIRUS STRAIN EDMONSTON	SCTVTREDGT	1510	P08362.1
628	RUBELLA VIRUS	SDAACWGFPTDTVMS	1511	BAA28178.1
629	MEASLES VIRUS STRAIN EDMONSTON	SDARAAHLPTGTP	1512	P04851.1
630	RUBELLA VIRUS STRAIN THERIEN	SDWHQGTHVCHTKHMDFWCVEHD	1513	P07566.1
631	MEASLES VIRUS STRAIN EDMONSTON	SELRRWIKYTQQRRV	1514	P04851.1
632	MEASLES VIRUS STRAIN EDMONSTON	SELRRWIKYTQQRRVVGEFR	1515	P04851.1
633	MEASLES VIRUS STRAIN EDMONSTON	SELSQL	1516	P08362.1
634	MEASLES VIRUS STRAIN EDMONSTON	SELSQLS	1517	P08362.1
635	BORDETELLA PERTUSSIS	SEYLAHRRIPPENIRRVTRV	1518	CAD44970.1
636	MEASLES VIRUS STRAIN EDMONSTON	SFLQGDQSENELP	1519	P04851.1
637	MEASLES VIRUS STRAIN EDMONSTON	SGKGVSFQLVKLGVW	1520	P08362.1
638	MEASLES VIRUS STRAIN EDMONSTON	SHRGVIADNQAKWAV	1521	P08362.1
639	MEASLES VIRUS STRAIN EDMONSTON	SIEHQVKDVLTPLFK	1522	P08362.1
640	MEASLES VIRUS STRAIN EDMONSTON	SKIGVVGIGSASYKV	1523	P69353.1
641	MEASLES MORBILLIVIRUS	SKRSELSQLSMYRVF	1524	P06830.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
642	MEASLES MORBILLIVIRUS	SLFVESPGQLIQRITDDPDVS	1525	ABI54110.1
643	MEASLES VIRUS STRAIN EDMONSTON	SLSTNLDVTNSIEHQ	1526	P08362.1
644	MEASLES VIRUS STRAIN EDMONSTON	SLSTNLDVTNSIEHQVKDVLTPLFK	1527	P08362.1
645	MEASLES VIRUS STRAIN EDMONSTON	SLWGSGLLML	1528	BAE98296.1
646	MEASLES VIRUS STRAIN EDMONSTON	SMKGLSSTSIVYILI	1529	P69353.1
647	MEASLES MORBILLIVIRUS	SMYRVFEVGV	1530	P08362.1
648	MEASLES MORBILLIVIRUS	SNDLSNCMVALGELK	1531	P06830.1
649	MEASLES VIRUS STRAIN HALLE	SPGQLIQR	1532	P10050.1
650	MEASLES VIRUS STRAIN EDMONSTON	SQDPQDSRRSAEP	1533	P04851.1
651	MEASLES MORBILLIVIRUS	SRIVINREHLMIDRP	1534	P06830.1
652	MEASLES VIRUS STRAIN EDMONSTON	SRKNKAQTRTPLQ	1535	P04851.1
653	RUBELLA VIRUS	SRLVGATP	1536	CAJ88851.1
654	RUBELLA VIRUS	SRLVGATPERPRLRLVDADDPLLR	1537	CAJ88851.1
655	MEASLES VIRUS STRAIN EDMONSTON	SRPGLKPDLTGTSKS	1538	P69353.1
656	BORDETELLA PERTUSSIS	SRRSVASIVGTLVRM	1539	CAD44970.1
657	RUBELLA VIRUS	SRWGLGSPNCHGPDW	1540	BAA28178.1
658	BORDETELLA PERTUSSIS	SSATK	1541	ACI16088.1
659	RUBELLA VIRUS	SSGGYAQLASYFNPG	1542	BAA28178.1
660	BORDETELLA PERTUSSIS	SSLGNGV	1543	ACI16083.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
661	MEASLES MORBILLIVIRUS	SSNLVILPGQDLQYV	1544	P06830.1
662	MEASLES VIRUS STRAIN EDMONSTON	SSQDPQDSRRSAEPL	1545	P04851.1
663	MEASLES VIRUS STRAIN EDMONSTON	SSQDPQDSRRSAEPLLSCKP	1546	P04851.1
664	MEASLES VIRUS STRAIN EDMONSTON	SSRRHKRFAGVVLAG	1547	P69353.1
665	MEASLES VIRUS STRAIN EDMONSTON	SSTSIVYILIAVCLG	1548	P69353.1
666	BORDETELLA PERTUSSIS	STPGIVI	1549	AAA83981.1
667	BORDETELLA PERTUSSIS	STPGIVIPPQEQITQHGSPYGRC	1550	AAA83981.1
668	BORDETELLA PERTUSSIS	STSSSRRYTEVY	1551	P04977.1
669	MEASLES VIRUS STRAIN EDMONSTON	SYFIVLSIAYPTLSE	1552	P69353.1
670	MEASLES VIRUS STRAIN EDMONSTON	SYRETGPSRASDA	1553	P04851.1
671	BORDETELLA PERTUSSIS	SYVK	1554	ACI16083.1
672	RUBELLA VIRUS	SYVQHPHKTVRVKFH	1555	BAA28178.1
673	RUBELLA VIRUS	TAPGPGEV	1556	CAJ88851.1
674	BORDETELLA PERTUSSIS	TATRLLSSTNSRLC	1557	AAA83981.1
675	MEASLES MORBILLIVIRUS	TDDPVIDRLYLSSHR	1558	P06830.1
676	MEASLES VIRUS STRAIN EDMONSTON	TEILSLFGPSLRDPI	1559	P69353.1
677	RUBELLA VIRUS	TETRTVWQLSVAGVS	1560	BAA28178.1
678	BORDETELLA PERTUSSIS	TEVYLEHRMQEAVE	1561	P04977.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
679	MEASLES VIRUS STRAIN EDMONSTON	TFMPEGTVCSQNALY	1562	P69353.1
680	RUBELLA VIRUS	TGACICEIPTDVSCE	1563	BAA28178.1
681	BORDETELLA PERTUSSIS	TGDLRAY	1564	ACI16083.1
682	MEASLES MORBILLIVIRUS	TGMQSWVPLSTDDPV	1565	P06830.1
683	RUBELLA VIRUS	TGNQQSRWGLGSPNC	1566	BAA28178.1
684	MEASLES VIRUS STRAIN EDMONSTON	TGPSRASDARAAH	1567	P04851.1
685	MEASLES MORBILLIVIRUS	TGTIINQDPDKILTY	1568	AAF02706.1
686	RUBELLA VIRUS	TGVVYGTHTTAVSET	1569	BAA28178.1
687	MEASLES VIRUS STRAIN EDMONSTON	TIRGQFSNMSLSLLD	1570	P08362.1
688	RUBELLA VIRUS	TLGATCALPLAGLLA	1571	BAA28178.1
689	MEASLES MORBILLIVIRUS	TLLNNCTRV	1572	P26031.1
690	MEASLES VIRUS STRAIN EDMONSTON-B	TLNVPPPPDPGR	1573	P03422.1
691	MEASLES VIRUS STRAIN EDMONSTON-B	TLNVPPPPDPGRASTSGTPIKK	1574	P03422.1
692	MEASLES MORBILLIVIRUS	TMTSQGMYGGTYPVE	1575	P06830.1
693	MEASLES VIRUS STRAIN EDMONSTON	TNLGNAIAKLEDAKE	1576	P69353.1
694	MEASLES VIRUS STRAIN EDMONSTON	TNMEDEADQYFSHDD	1577	P04851.1
695	MEASLES VIRUS STRAIN EDMONSTON	TNMEDEADQYFSHDDPISSD	1578	P04851.1
696	RUBELLA VIRUS STRAIN M33	TNPFQAAVARGLRPP	1579	CAA28880.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
697	MEASLES MORBILLIVIRUS	TNSIEHQVKDVLTPL	1580	P06830.1
698	MEASLES VIRUS STRAIN EDMONSTON	TNYLEQPVSNDLSNC	1581	P08362.1
699	MEASLES MORBILLIVIRUS	TNYLEQPVSNDLSNCMVALGELKLAAL	1582	AAR89413.1
700	RUBELLA VIRUS	TPERPRLR	1583	CAJ88851.1
701	RUBELLA VIRUS STRAIN THERIEN	TPERPRLRLVDADDPLLRTA	1584	P07566.1
702	MEASLES VIRUS STRAIN HALLE	TPGNKPRIA	1585	P10050.1
703	MEASLES VIRUS STRAIN EDMONSTON	TPLDIDTASESSQDP	1586	P04851.1
704	MEASLES VIRUS STRAIN EDMONSTON	TPLDIDTASESSQDPQDSRR	1587	P04851.1
705	MEASLES VIRUS STRAIN EDMONSTON	TPLFKIIGDEVGLRT	1588	P08362.1
706	MEASLES VIRUS STRAIN EDMONSTON	TPLQCTM	1589	P04851.1
707	RUBELLA VIRUS	TPVIGSQA	1590	CAJ88851.1
708	RUBELLA VIRUS	TPVIGSQARK	1591	BAA19893.1
709	MEASLES VIRUS STRAIN EDMONSTON	TQGYLISNFDESSCT	1592	P69353.1
710	BORDETELLA PERTUSSIS	TRANPNPYTSRRSVASIVGTLVRM	1593	P04977.1
711	RUBELLA VIRUS	TRFGCAMRWGLPP	1594	NP_740663.1
712	BORDETELLA PERTUSSIS	TRNTGQPATDHYYSNV	1595	AAA83981.1
713	MEASLES VIRUS STRAIN EDMONSTON	TRTPLQCTMTEIF	1596	P04851.1
714	RUBELLA VIRUS	TRWHRLLRMPVR	1597	ABD64216.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
715	MEASLES VIRUS STRAIN EDMONSTON	TSGSGGAIRGIKHII	1598	P04851.1
716	MEASLES VIRUS STRAIN EDMONSTON	TSGSGGAIRGIKHIIIVPIP	1599	P04851.1
717	MEASLES VIRUS STRAIN EDMONSTON	TSQGMYGGTYLVEKP	1600	P08362.1
718	MEASLES MORBILLIVIRUS	TSRVEHAVVYYVYSP	1601	P06830.1
719	BORDETELLA PERTUSSIS	TSSSRRYTEVYL	1602	ACI04548.1
720	BORDETELLA PERTUSSIS	TSYVG	1603	ACI16088.1
721	MEASLES VIRUS STRAIN EDMONSTON	TTEDKISRAVGPRQA	1604	P04851.1
722	MEASLES VIRUS STRAIN EDMONSTON	TTEDKISRAVGPRQAQVSFL	1605	P04851.1
723	RUBELLA VIRUS STRAIN M33	TTERIETRSARHP	1606	ABD64214.1
724	MEASLES VIRUS STRAIN EDMONSTON	TTNQAIEAIRQAGQE	1607	P69353.1
725	RUBELLA VIRUS	TTSDPWHPPGPLGLK	1608	BAA28178.1
726	MEASLES VIRUS STRAIN EDMONSTON	TVCSQNALYPMSPLL	1609	P69353.1
727	RUBELLA VIRUS	TVNGEDVGAVPPGKF	1610	BAA28178.1
728	MEASLES MORBILLIVIRUS	TYPVEKPNLSSKRSE	1611	P06830.1
729	RUBELLA VIRUS	VAGVSCNVTTEHPFC	1612	BAA28178.1
730	BORDETELLA PERTUSSIS	VAPGIVIPPKALFTQQGGAYGRC	1613	P04979.1
731	RUBELLA VIRUS	VCHTKHMDFWCVEHDRPPPATPTPL	1614	NP_740663.1
732	RUBELLA VIRUS	VCQRHSPDCSRLVGATPER	1615	BAA19893.1
733	RUBELLA VIRUS	VDADDPLL	1616	CAJ88851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
734	RUBELLA VIRUS	VDADDPLLRTAPGPGEVWVT	1617	BAA19893.1
735	CORYNEBACTERIUM DIPHTHERIAE	VDIGFAAYNFVESIINLFQV	1618	AAV70486.1
736	MEASLES VIRUS STRAIN EDMONSTON	VEIAEYRRLLRTVLE	1619	P69353.1
737	MEASLES VIRUS STRAIN EDMONSTON	VELENSMGGLNFGRS	1620	P04851.1
738	MEASLES VIRUS STRAIN EDMONSTON	VELENSMGGLNFGRSYFDPA	1621	P04851.1
739	MEASLES MORBILLIVIRUS	VELKIKIASGFGPLI	1622	P06830.1
740	RUBELLA VIRUS	VEMDEWIHAHTTSD	1623	SRC265968
741	RUBELLA VIRUS	VEMPEWIHAHTTSDP	1624	BAA28178.1
742	CORYNEBACTERIUM DIPHTHERIAE	VERRLVKVL	1625	P33120.2
743	MEASLES MORBILLIVIRUS	VESPGQLI	1626	ABI54110.1
744	MEASLES VIRUS STRAIN EDMONSTON	VESPGQLIQRITDDP	1627	P04851.1
745	MEASLES VIRUS STRAIN EDMONSTON	VESPGQLIQRITDDPDVSIR	1628	P04851.1
746	RUBELLA VIRUS	VFALASYVQHPHKTV	1629	BAA28178.1
747	RUBELLA VIRUS	VGATPERP	1630	CAJ88851.1
748	RUBELLA VIRUS	VGATPERPRL	1631	BAA19893.1
749	RUBELLA VIRUS	VGATPERPRLRLVDA	1632	BAA28178.1
750	MEASLES VIRUS STRAIN EDMONSTON	VGIGSASYKVMTRSS	1633	P69353.1
751	MEASLES VIRUS STRAIN EDMONSTON	VGLRTPQRFTDLVKF	1634	P08362.1
752	CORYNEBACTERIUM DIPHTHERIAE	VHHNTEEIVAQSIALSSLMV	1635	AAV70486.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
753	MEASLES VIRUS STRAIN EDMONSTON	VHRLEGVSYNIGSQE	1636	P69353.1
754	RUBELLA VIRUS	VIGSQARK	1637	CAJ88851.1
755	BORDETELLA PERTUSSIS	VITGSI	1638	ACI16088.1
756	BORDETELLA PERTUSSIS	VITGTI	1639	ACI16083.1
757	MEASLES VIRUS STRAIN EDMONSTON	VKQSRGEA	1640	P04851.1
758	MEASLES VIRUS STRAIN EDMONSTON	VLFVMFLSLI	1641	P08362.1
759	MEASLES MORBILLIVIRUS	VLFVMFLSLIGLLAI	1642	P06830.1
760	MEASLES MORBILLIVIRUS	VLTPLFKIIGDEVGL	1643	P06830.1
761	MEASLES VIRUS STRAIN EDMONSTON	VMTRSSHQSLVIKLM	1644	P69353.1
762	RUBELLA VIRUS	VPAAPCARIWNGTQR	1645	BAA28178.1
763	RUBELLA VIRUS	VPPDPGDLVEYIMNY	1646	BAA28178.1
764	MEASLES VIRUS STRAIN EDMONSTON	VQSVASSRRHKRFAG	1647	P69353.1
765	BORDETELLA PERTUSSIS	VQTGGTSRTVTMRYLAS	1648	ACI16083.1
766	BORDETELLA PERTUSSIS	VQVRI	1649	ACI16083.1
767	RUBELLA VIRUS	VRAYNQPAGDV	1650	NP_740662.1
768	RUBELLA VIRUS	VRFESKIVDGGCFAP	1651	BAA28178.1
769	RUBELLA VIRUS	VRLAGVRFESKIVDG	1652	BAA28178.1
770	MEASLES VIRUS STRAIN EDMONSTON	VSGSFGNRFILSQGN	1653	P69353.1
771	BORDETELLA PERTUSSIS	VSKEEQYYDYEDAT	1654	AAA83981.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
772	MEASLES VIRUS STRAIN EDMONSTON	VSKGNCSGPTTIRGQ	1655	P08362.1
773	RUBELLA VIRUS	VTAALLNTPPPYQVS	1656	BAA28178.1
774	RUBELLA VIRUS	VTGCYQCGTPALVEG	1657	BAA28178.1
775	RUBELLA VIRUS	VTPVIGSQ	1658	CAJ88851.1
776	RUBELLA VIRUS STRAIN THERIEN	VTTEHPFCNTPHGQLEVQVPPD	1659	P07566.1
777	MEASLES VIRUS STRAIN EDMONSTON	VVLAGAALGVATAAQ	1660	P69353.1
778	RUBELLA VIRUS	VWQLSVAGVSCNVTT	1661	BAA28178.1
779	RUBELLA VIRUS	VWVTPVIG	1662	CAJ88851.1
780	RUBELLA VIRUS	VWVTPVIGSQAR	1663	BAA19893.1
781	MEASLES VIRUS STRAIN EDMONSTON	VYILIAVCLGGLIGI	1664	P69353.1
782	MEASLES VIRUS STRAIN EDMONSTON	VYLHRIDLGPPISLE	1665	P69353.1
783	BORDETELLA PERTUSSIS	VYRYDSRP	1666	P04977.1
784	BORDETELLA PERTUSSIS	VYRYDSRPPEDV	1667	P04977.1
785	MEASLES MORBILLIVIRUS	VYWLTIPPMKNLALG	1668	P06830.1
786	RUBELLA VIRUS	WDLEATGACICEIPT	1669	BAA28178.1
787	MEASLES MORBILLIVIRUS	WDQKLWCRHFCVLAD	1670	AAR89413.1
788	RUBELLA VIRUS	WGFPTDTVMSVFALA	1671	BAA28178.1
789	RUBELLA VIRUS	WHPPGPLGLKFKTVR	1672	BAA28178.1
790	RUBELLA VIRUS	WIHAHTTSDPWHPPG	1673	BAA28178.1
791	MEASLES VIRUS STRAIN EDMONSTON	WLTIPPMKNLALGVI	1674	P08362.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
792	MEASLES VIRUS STRAIN EDMONSTON	WQESRKNKAQTRTPLQCTMT	1675	P04851.1
793	RUBELLA VIRUS	WVCIFMVCRRACR	1676	SRC265968
794	RUBELLA VIRUS	WVTPVIGS	1677	CAJ88851.1
795	MEASLES VIRUS STRAIN EDMONSTON	WYTTVPKYVATQGYL	1678	P69353.1
796	MEASLES VIRUS STRAIN EDMONSTON	YALGGDINKVLEKLG	1679	P69353.1
797	MEASLES VIRUS STRAIN EDMONSTON	YAMGVGVELE	1680	P04851.1
798	MEASLES MORBILLIVIRUS	YAMGVGVELEN	1681	ABI54110.1
799	MEASLES MORBILLIVIRUS	YCADVAAEELMNALV	1682	AAR89413.1
800	MEASLES MORBILLIVIRUS	YDFRDLTWCINPPER	1683	P06830.1
801	BORDETELLA PERTUSSIS	YFEPGPT	1684	ACI16083.1
802	RUBELLA VIRUS	YFNPGGSYYKQYHPT	1685	BAA28178.1
803	MEASLES VIRUS STRAIN EDMONSTON	YFRLGQEMVRRSAGK	1686	P04851.1
804	MEASLES VIRUS STRAIN EDMONSTON	YFRLGQEMVRRSAGKVSSTL	1687	P04851.1
805	MEASLES MORBILLIVIRUS	YFYPFRLPIKGVPIE	1688	P06830.1
806	BORDETELLA PERTUSSIS	YGDNAGRILAGALAT	1689	P04977.1
807	RUBELLA VIRUS	YGHATVEMPEWIHAH	1690	BAA28178.1
808	RUBELLA VIRUS	YIMNYTGNQQSRWGL	1691	BAA28178.1
809	MEASLES VIRUS STRAIN EDMONSTON	YINNELIPSMNQLSC	1692	P69353.1
810	RUBELLA VIRUS	YLCTAPGCATQAPVP	1693	BAA28178.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
811	MEASLES MORBILLIVIRUS	YLFTVPIKEAGEDCH	1694	P06830.1
812	MEASLES VIRUS STRAIN EDMONSTON	YLHDPEFNL	1695	ABK40531.1
813	MEASLES VIRUS STRAIN EDMONSTON	YLNMSRLFV	1696	ABK40531.1
814	MEASLES VIRUS STRAIN HALLE	YPALGLHEF	1697	P10050.1
815	MEASLES MORBILLIVIRUS	YPALGLHEFA	1698	ABI54110.1
816	MEASLES VIRUS STRAIN EDMONSTON	YPALGLHEFAGELST	1699	P04851.1
817	MEASLES VIRUS STRAIN EDMONSTON	YPALGLHEFAGELSTLESLM	1700	P04851.1
818	MEASLES VIRUS STRAIN EDMONSTON	YPFRLPIKGVPIELQ	1701	P08362.1
819	MEASLES VIRUS STRAIN HALLE	YPLLWSYAM	1702	P10050.1
820	MUMPS RUBULAVIRUS	YQQQGRL	1703	P21186.1
821	BORDETELLA PERTUSSIS	YQSEYLAHRR	1704	P04977.1
822	MEASLES VIRUS STRAIN EDMONSTON	YRETGPSRASDARAA	1705	P04851.1
823	MEASLES VIRUS STRAIN EDMONSTON	YRETGPSRASDARAAHLPTG	1706	P04851.1
824	RUBELLA VIRUS	YRNASDVLPGHWLQGGWGCYNLSDW	1707	NP_740663.1
825	MEASLES VIRUS STRAIN EDMONSTON	YRRLLRTVLEPIRDA	1708	P69353.1
826	BORDETELLA PERTUSSIS	YRYDSRPP	1709	P04977.1
827	MEASLES VIRUS STRAIN EDMONSTON	YSGGDLLGILESRGI	1710	P69353.1
828	BORDETELLA PERTUSSIS	YSKVTATBLLASTNSRLCAVFVRDG	1711	SRC280066

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
829	MEASLES VIRUS STRAIN EDMONSTON	YSPSRSFSYFYPFRL	1712	P08362.1
830	RUBELLA VIRUS STRAIN THERIEN	YTGNQQSRWGLGSPNCHGPDWASPV	1713	P07566.1
831	MEASLES MORBILLIVIRUS	YVLLAVLFV	1714	P08362.1
832	MEASLES MORBILLIVIRUS	YVYSPGRSFSYFYPF	1715	P06830.1
833	BORDETELLA PERTUSSIS	YYDYEDATFQTYALTGISLCNPAASIC	1716	P04979.1
834	MEASLES MORBILLIVIRUS	GDLLGILESRGIKAR	1717	AAF02706.1
835	MEASLES MORBILLIVIRUS	TVPKYVATQGYLISN	1718	AAL29688.1
836	MEASLES MORBILLIVIRUS	KPWDSPQEI	1719	P26035.1
837	MEASLES MORBILLIVIRUS	KPWESPQEI	1720	CAA34579.1
838	BORDETELLA PERTUSSIS	ATYQSEYLAHRRIPP	1721	ACI04548.1
839	BORDETELLA PERTUSSIS	CMARQAESSEAMAAWSERAGEAMVLVYYESIAYSF	1722	ACI04548.1
840	BORDETELLA PERTUSSIS	CQVGSSNSAFVSTSSSRRYTEVYL	1723	ACI04548.1
841	BORDETELLA PERTUSSIS	DDPPATVYRYDSRPP	1724	ACI04548.1
842	BORDETELLA PERTUSSIS	GALATYQSEYLAHRRIPP	1725	ACI04548.1
843	BORDETELLA PERTUSSIS	MAAWSERAGEAMVLVYYESIAYSF	1726	ACI04548.1
844	BORDETELLA PERTUSSIS	MVLVYYESIAYSF	1727	ACI04548.1
845	BORDETELLA PERTUSSIS	PATVYRYDSRPPEDV	1728	ACI04548.1
846	BORDETELLA PERTUSSIS	YDSRPPEDV	1729	ACI04548.1
847	BORDETELLA PERTUSSIS	EPGITTNYDT	1730	ACI16087.1
848	BORDETELLA PERTUSSIS	GDLRAYKMVYATNPQTQLSN	1731	ACI16083.1
849	BORDETELLA PERTUSSIS	KNGDVEASAITTYVGFSVVYP	1732	ACI16083.1
850	BORDETELLA PERTUSSIS	KVTNGSKSYTLRYLASYVK	1733	ACI16088.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
851	BORDETELLA PERTUSSIS	QALGALKLYFEPGITTNYDTGDLIAY KQTYNASGN	1734	ACI16088 .1
852	BORDETELLA PERTUSSIS	YATNPQTQLS	1735	ACI16083 .1
853	CORYNEBACTERI UM DIPHTHERIAE	DNENPLSGKAGGVVKVTYPGLTKV	1736	AAV7048 6.1
854	CORYNEBACTERI UM DIPHTHERIAE	ENFSSYHGTKPGYVDSI	1737	AAV7048 6.1
855	CORYNEBACTERI UM DIPHTHERIAE	KVDNAETIKKELGLSLTEP	1738	AAV7048 6.1
856	RUBELLA VIRUS STRAIN M33	MEDLQKALEAQSRALRAGLAA	1739	CAA2888 0.1
857	CORYNEBACTERI UM DIPHTHERIAE	QKGIQKPKSGTQGNYDDDWKGFY	1740	AAV7048 6.1
858	RUBELLA VIRUS STRAIN M33	RTGAWQRKDWSRAPPPPEERQESRS QTPAPKPSR	1741	CAA2888 0.1
859	RUBELLA VIRUS	AAGASQSRRPRPPRHARAQHLPEMT PAVT	1742	SRC2659 68
860	RUBELLA VIRUS	CVTSWLWSEGEGAVFYRVDLHFINL GTP	1743	CAA2888 0.1
861	RUBELLA VIRUS	FRVGGTRWHRLLRMPVRGLDGDTAP LP	1744	CAA2888 0.1
862	CORYNEBACTERI UM DIPHTHERIAE	GRKIRMRCRAIDGDVTFCRPKSPVYV GN	1745	1007216A
863	RUBELLA VIRUS	GTPPLDEDGRWDPALMYNPCGPEPPA HV	1746	CAA2888 0.1
864	CORYNEBACTERI UM DIPHTHERIAE	GVHANLHVAFHRSSSEKIHSNEISSDS IGVLGYQKTVDHTKVNSKLSLFFEIK S	1747	AAV7048 6.1
865	RUBELLA VIRUS	MASTTPITMEDLQKALEAQSRALRA GLAAG	1748	ABD6420 0.1
866	RUBELLA VIRUS	PELGPPTNPFQAAVARGLRPPLHDPD TEAPTEAC	1749	CAA2888 0.1
867	RUBELLA VIRUS	PLPPHTTERIETRSARHPWRIRFGAP	1750	CAA2888 0.1
868	RUBELLA VIRUS	SRAPPPPEERQESRSQTPAPKPSRAPP	1751	CAA2888 0.1
869	RUBELLA VIRUS	SRAPPQQPQPPRMQTGRGGSAPRPEL GP	1752	CAA2888 0.1
870	RUBELLA VIRUS	TPAVTPEGPAPPRTGAWQRKDWSRAP P	1753	CAA2888 0.1
871	RUBELLA VIRUS	VRAYNQPAGDVRGVWGKGERTYAE QDFRV	1754	CAA2888 0.1
872	RUBELLA VIRUS	AFGHSDAACWGFPTDTVMSV	1755	CAA2888 0.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
873	RUBELLA VIRUS	CARIWNGTQRACTFWAVNAYS	1756	CAA28880.1
874	RUBELLA VIRUS	EEAFTYLCTAPGCATQTPVPVR	1757	CAA28880.1
875	RUBELLA VIRUS	FAPWDLEATGACICEIPTDV	1758	CAA28880.1
876	RUBELLA VIRUS	GEDVGAFPPGKFVTAAL	1759	CAA28880.1
877	RUBELLA VIRUS	GEVWVTPVIGSQARKCGLHI	1760	CAA28880.1
878	RUBELLA VIRUS	GQLEVQVPPDPGDLVEYIMN	1761	CAA28880.1
879	RUBELLA VIRUS	GSYYKQYHPTACEVEPAFGH	1762	CAA28880.1
880	RUBELLA VIRUS	IHAHTTSDPWHPPGPLGLKF	1763	CAA28880.1
881	RUBELLA VIRUS	IMNYTGNQQSRWGLGSPNCH	1764	CAA28880.1
882	RUBELLA VIRUS	LHIRAGPYGHATVEMPEWIH	1765	CAA28880.1
883	RUBELLA VIRUS	LKFKTVRPVALPRALAPPRN	1766	CAA28880.1
884	RUBELLA VIRUS	LNTPPPYQVSCGGESDRASAGH	1767	CAA28880.1
885	RUBELLA VIRUS	NCHGPDWASPVCQRHSPDCS	1768	CAA28880.1
886	RUBELLA VIRUS	PDCSRLVGATPERPRLRLVD	1769	CAA28880.1
887	RUBELLA VIRUS	PRNVRVTGCYQCGTPALVEG	1770	CAA28880.1
888	RUBELLA VIRUS	PTDVSCEGLGAWVPTAPCARI	1771	CAA28880.1
889	RUBELLA VIRUS	RLVDADDPLLRTAPGPGEVW	1772	CAA28880.1
890	RUBELLA VIRUS	SVFALASYVQHPHKTVRVKF	1773	CAA28880.1
891	RUBELLA VIRUS	VEGLAPGGGNCHLTVNGEDV	1774	CAA28880.1
892	RUBELLA VIRUS	VKFHTETRTVWQLSVAGVSC	1775	CAA28880.1
893	RUBELLA VIRUS	VPVRLAGVGFESKIVDGGCF	1776	CAA28880.1
894	RUBELLA VIRUS	VSCNVTTEHPFCNTPHGQLE	1777	CAA28880.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
895	BORDETELLA PERTUSSIS	AAASSPDAHVPF	1778	AAA22983.1
896	BORDETELLA PERTUSSIS	AASSPDA	1779	AAA22983.1
897	BORDETELLA PERTUSSIS	AKLGAAASSPDA	1780	AAA22983.1
898	BORDETELLA PERTUSSIS	AMKPYEVTPTRM	1781	AAA22983.1
899	BORDETELLA PERTUSSIS	AMTHLSPALADVPYVLVKTNMVVTS	1782	AAA22983.1
900	BORDETELLA PERTUSSIS	ASSPDAHVPFCF	1783	AAA22983.1
901	BORDETELLA PERTUSSIS	ASSPDAHVPFCFGKDLKRPGSSPME	1784	AAA22983.1
902	BORDETELLA PERTUSSIS	CFGKDLKRPGSS	1785	AAA22983.1
903	BORDETELLA PERTUSSIS	CFGKDLKRPGSSPMEVMLRAVFMQQ	1786	AAA22983.1
904	BORDETELLA PERTUSSIS	CGIAAKLGAAAS	1787	AAA22983.1
905	BORDETELLA PERTUSSIS	CGIAAKLGAAASSPDAHVPFCFGKD	1788	AAA22983.1
906	BORDETELLA PERTUSSIS	DAHVPFCFGKDL	1789	AAA22983.1
907	BORDETELLA PERTUSSIS	DLKRPGSSPMEV	1790	AAA22983.1
908	BORDETELLA PERTUSSIS	DVPYVLVKTNMV	1791	AAA22983.1
909	BORDETELLA PERTUSSIS	DVPYVLVKTNMVVTSVAMKPYEVTPT	1792	AAA22983.1
910	BORDETELLA PERTUSSIS	EVMLRAVFMQQR	1793	AAA22983.1
911	BORDETELLA PERTUSSIS	FEGKPALELIRM	1794	AAA22983.1
912	BORDETELLA PERTUSSIS	FLGPKQLTFEGK	1795	AAA22983.1
913	BORDETELLA PERTUSSIS	FLGPKQLTFEGKPALELIRMVECSG	1796	AAA22983.1
914	BORDETELLA PERTUSSIS	FMQQRPLRMFLGPKQLT	1797	AAA22983.1
915	BORDETELLA PERTUSSIS	GKDLKRPGSSPM	1798	AAA22983.1
916	BORDETELLA PERTUSSIS	GKDLKRPGSSPME	1799	AAA22983.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
917	BORDETELLA PERTUSSIS	GKPALELIRMVE	1800	AAA22983.1
918	BORDETELLA PERTUSSIS	GPKQLTFEGKPA	1801	AAA22983.1
919	BORDETELLA PERTUSSIS	HVPFCFGKDLKR	1802	AAA22983.1
920	BORDETELLA PERTUSSIS	IAAKLGAAASSP	1803	AAA22983.1
921	BORDETELLA PERTUSSIS	KPYEVTPTRMLV	1804	AAA22983.1
922	BORDETELLA PERTUSSIS	KQLTFEGKPALE	1805	AAA22983.1
923	BORDETELLA PERTUSSIS	KRPGSSPMEVML	1806	AAA22983.1
924	BORDETELLA PERTUSSIS	LELIRMVECSGK	1807	AAA22983.1
925	BORDETELLA PERTUSSIS	LGAAASSPDAHV	1808	AAA22983.1
926	BORDETELLA PERTUSSIS	LIRMVECSGKQD	1809	AAA22983.1
927	BORDETELLA PERTUSSIS	LVCGIAAKLGAA	1810	AAA22983.1
928	BORDETELLA PERTUSSIS	MKPYEVTPTRM	1811	AAA22983.1
929	BORDETELLA PERTUSSIS	MLRAVFMQQRPL	1812	AAA22983.1
930	BORDETELLA PERTUSSIS	MQQRPLRM	1813	AAA22983.1
931	BORDETELLA PERTUSSIS	MQQRPLRMFLGP	1814	AAA22983.1
932	BORDETELLA PERTUSSIS	MVVTSVAMKPYE	1815	AAA22983.1
933	BORDETELLA PERTUSSIS	MVVTSVAMKPYEVTPTRMLVCGIAA	1816	AAA22983.1
934	BORDETELLA PERTUSSIS	PALELIRMVECS	1817	AAA22983.1
935	BORDETELLA PERTUSSIS	PALELIRMVECSGK	1818	AAA22983.1
936	BORDETELLA PERTUSSIS	PFCFGKDLKRPG	1819	AAA22983.1
937	BORDETELLA PERTUSSIS	PGSSPMEVMLRA	1820	AAA22983.1
938	BORDETELLA PERTUSSIS	PGSSPMEVMLRAVF	1821	AAA22983.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
939	BORDETELLA PERTUSSIS	PKQLTFEGK	1822	AAA22983.1
940	BORDETELLA PERTUSSIS	PLRMFLGPKQLT	1823	AAA22983.1
941	BORDETELLA PERTUSSIS	PTRMLVCGIAAK	1824	AAA22983.1
942	BORDETELLA PERTUSSIS	PYVLVKTNMVVT	1825	AAA22983.1
943	BORDETELLA PERTUSSIS	QLTFEGKPALELIRMVECSGKQDCP	1826	AAA22983.1
944	BORDETELLA PERTUSSIS	QRPLRMFLGPKQ	1827	AAA22983.1
945	BORDETELLA PERTUSSIS	RAVFMQQRPLRM	1828	AAA22983.1
946	BORDETELLA PERTUSSIS	RMFLGPKQLTFE	1829	AAA22983.1
947	BORDETELLA PERTUSSIS	RMLVCGIAAKLG	1830	AAA22983.1
948	BORDETELLA PERTUSSIS	RMVECSGKQDCP	1831	AAA22983.1
949	BORDETELLA PERTUSSIS	SPDAHVPFCFGK	1832	AAA22983.1
950	BORDETELLA PERTUSSIS	SSPMEVMLRAVF	1833	AAA22983.1
951	BORDETELLA PERTUSSIS	SSPMEVMLRAVFMQQRPLRMFLGPK	1834	AAA22983.1
952	BORDETELLA PERTUSSIS	SVAMKPYEVTPT	1835	AAA22983.1
953	BORDETELLA PERTUSSIS	VECSGKQDCP	1836	AAA22983.1
954	BORDETELLA PERTUSSIS	VFMQQRPLRMFL	1837	AAA22983.1
955	BORDETELLA PERTUSSIS	VFMQQRPLRMFLGPKQLTFEGKPAL	1838	AAA22983.1
956	BORDETELLA PERTUSSIS	VKTNMVVTSVAM	1839	AAA22983.1
957	BORDETELLA PERTUSSIS	VLVKTNMVVTSV	1840	AAA22983.1
958	BORDETELLA PERTUSSIS	VTPTRMLVCGIA	1841	AAA22983.1
959	BORDETELLA PERTUSSIS	VTSVAMKPYEVT	1842	AAA22983.1
960	BORDETELLA PERTUSSIS	YEVTPTRMLVCG	1843	AAA22983.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
961	BORDETELLA PERTUSSIS	YEVTPTRMLVCGIAAKLGAAASSPD	1844	AAA22983.1
962	BORDETELLA PERTUSSIS	CASPYEGRYRDMYDALR	1845	P04979.1
963	BORDETELLA PERTUSSIS	CAVFVRDGQSV	1846	P04979.1
964	BORDETELLA PERTUSSIS	CITTIYKTG	1847	P04979.1
965	BORDETELLA PERTUSSIS	CPNGTRALTV	1848	P04979.1
966	BORDETELLA PERTUSSIS	DALRRLLYMIYMSG	1849	P04979.1
967	RUBELLA VIRUS STRAIN THERIEN	GNRGRGQRRDWSRAPPPPEERQETRS	1850	P07566.1
968	BORDETELLA PERTUSSIS	GQPAADHYYSKVT	1851	P04979.1
969	RUBELLA VIRUS	GSPNCHGPDWASPVCQRHS	1852	ABD64214.1
970	MEASLES VIRUS STRAIN EDMONSTON	HKSLSTNLDVTNSIEHQ	1853	P08362.1
971	BORDETELLA PERTUSSIS	LFTQQGGAYGRC	1854	P04979.1
972	MEASLES VIRUS STRAIN EDMONSTON	LIGLLAIAGIRLHRAAIYTAEI	1855	P08362.1
973	MEASLES MORBILLIVIRUS	PDTAADSELRRWIKY	1856	ABI54110.1
974	RUBELLA VIRUS STRAIN THERIEN	PNCHGPDWASPVCQRHS	1857	P07566.1
975	RUBELLA VIRUS	QTPAPKPSRAPPQQPQPPRMQTGR	1858	ABD64216.1
976	RUBELLA VIRUS VACCINE STRAIN RA27/3	RAGLTAGASQSRRPRPPR	1859	CAA33016.1
977	RUBELLA VIRUS VACCINE STRAIN RA27/3	RFGAPQAFLAGLLLAAVAVGTARA	1860	ABD64214.1
978	BORDETELLA PERTUSSIS	RGNAELQTYLRQITPG	1861	P04979.1
979	BORDETELLA PERTUSSIS	RVHVSKEEQYYDYED	1862	P04979.1
980	BORDETELLA PERTUSSIS	SIYGLYDGTYL	1863	P04979.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
981	BORDETELLA PERTUSSIS	SKVTATRLLASTNS	1864	P04979.1
982	BORDETELLA PERTUSSIS	TQQGGAYGRCPNGTRA	1865	P04979.1
983	BORDETELLA PERTUSSIS	VAPGIVIPPKAL	1866	P04979.1
984	BORDETELLA PERTUSSIS	DSRPPEDVFQNGFTAWG	1867	ACI04548.1
985	BORDETELLA PERTUSSIS	EHRMQEAVEAERAGR	1868	ACI04548.1
986	MEASLES VIRUS STRAIN EDMONSTON	ETCFQQACKGKIQALCENPEWA	1869	P08362.1
987	BORDETELLA PERTUSSIS	EYVDTYGDNAGRILAGALATYQ	1870	ACI04548.1
988	BORDETELLA PERTUSSIS	HRRIPPENIRRVTR	1871	ACI04548.1
989	BORDETELLA PERTUSSIS	MARQAESSE	1872	ACI04548.1
990	BORDETELLA PERTUSSIS	MQEAVEAERAGR	1873	ACI04548.1
991	BORDETELLA PERTUSSIS	SQQTRANPNPYTSRR	1874	ACI04548.1
992	BORDETELLA PERTUSSIS	TRANPNPYTSRRSVASIVGTLVHG	1875	SRC280066
993	BORDETELLA PERTUSSIS	TVYRYDSRPPED	1876	ACI04548.1
994	MEASLES MORBILLIVIRUS	NDRNLLD	1877	P10050.1
995	MEASLES MORBILLIVIRUS	NMEDEADQYFSHDDPISSDQSRFGWFENK	1878	P04851.1
996	MEASLES MORBILLIVIRUS	SRASDARAAHLPTGTPLDID	1879	P04851.1
997	BORDETELLA PERTUSSIS	EDVFQNGFTAW	1880	ACI04548.1
998	CORYNEBACTERIUM DIPHTHERIAE	AEGSSSVEYINNWEQAK	1881	AAV70486.1
999	CORYNEBACTERIUM DIPHTHERIAE	GPIKNKMSESPNKT	1882	AAV70486.1
1000	MEASLES VIRUS STRAIN HALLE	GPKLTGALIGILSLFVESPGQLIQRITDDPDV	1883	P10050.1
1001	CORYNEBACTERIUM DIPHTHERIAE	GYQKTVDHTKVNSK	1884	AAV70486.1
1002	CORYNEBACTERIUM DIPHTHERIAE	KTVDH	1885	AAV70486.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1003	CORYNEBACTERIUM DIPHTHERIAE	SESPNK	1886	AAV70486.1
1004	CORYNEBACTERIUM DIPHTHERIAE	AEGSSSVEYINNWEQAKALS	1887	AAV70486.1
1005	CORYNEBACTERIUM DIPHTHERIAE	AQAIPLVGELVDIGFAAYNF	1888	AAV70486.1
1006	CORYNEBACTERIUM DIPHTHERIAE	ASRVVLSLPFAEGSSSVEYI	1889	AAV70486.1
1007	CORYNEBACTERIUM DIPHTHERIAE	CINLDWDVIRDKTKTKIESL	1890	AAV70486.1
1008	CORYNEBACTERIUM DIPHTHERIAE	CRAIDGDVTFCRPKSPVYVG	1891	AAV70486.1
1009	CORYNEBACTERIUM DIPHTHERIAE	CRPKSPVYVGNGVHANLHVA	1892	AAV70486.1
1010	CORYNEBACTERIUM DIPHTHERIAE	DAAGYSVDNENPLSGKAGGV	1893	AAV70486.1
1011	CORYNEBACTERIUM DIPHTHERIAE	DKTKTKIESLKEHGPIKNKM	1894	AAV70486.1
1012	CORYNEBACTERIUM DIPHTHERIAE	EEFIKRFGDGASRVVLSLPF	1895	AAV70486.1
1013	CORYNEBACTERIUM DIPHTHERIAE	EKAKQYLEEFHQTALEHPEL	1896	AAV70486.1
1014	CORYNEBACTERIUM DIPHTHERIAE	FHRSSSEKIHSNEISSDSIG	1897	AAV70486.1
1015	CORYNEBACTERIUM DIPHTHERIAE	GADDVVDSSKSFVMENFSSY	1898	CAE11230.1
1016	CORYNEBACTERIUM DIPHTHERIAE	GKRGQDAMYEYMAQACAGNR	1899	AAV70486.1
1017	CORYNEBACTERIUM DIPHTHERIAE	GSVMGIADGAVHHNTEEIVA	1900	AAV70486.1
1018	CORYNEBACTERIUM DIPHTHERIAE	GTQGNYDDDWKGFYSTDNKY	1901	AAV70486.1
1019	CORYNEBACTERIUM DIPHTHERIAE	HDGYAVSWNTVEDSIIRTGF	1902	AAV70486.1
1020	CORYNEBACTERIUM DIPHTHERIAE	HGTKPGYVDSIQKGIQKPKS	1903	AAV70486.1
1021	CORYNEBACTERIUM DIPHTHERIAE	HQTALEHPELSELKTVTGTN	1904	AAV70486.1
1022	CORYNEBACTERIUM DIPHTHERIAE	IQKGIQKPKSGTQGNYDDDW	1905	AAV70486.1
1023	CORYNEBACTERIUM DIPHTHERIAE	KEHGPIKNKMSESPNKTVSE	1906	AAV70486.1
1024	CORYNEBACTERIUM DIPHTHERIAE	KGFYSTDNKYDAAGYSVDNE	1907	AAV70486.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1025	CORYNEBACTERIUM DIPHTHERIAE	LDVNKSKTHISVNGRKIRMR	1908	AAV70486.1
1026	RUBELLA VIRUS	MASTIPITMEDLQKALEA	1909	SRC265968
1027	CORYNEBACTERIUM DIPHTHERIAE	NGVHANLHVAFHRSSSEKIH	1910	AAV70486.1
1028	CORYNEBACTERIUM DIPHTHERIAE	NNWEQAKALSVELEINFETR	1911	AAV70486.1
1029	CORYNEBACTERIUM DIPHTHERIAE	NPLSGKAGGVVKVTYPGLTK	1912	AAV70486.1
1030	CORYNEBACTERIUM DIPHTHERIAE	PVFAGANYAAWAVNVAQVID	1913	AAV70486.1
1031	CORYNEBACTERIUM DIPHTHERIAE	SELKTVTGTNPVFAGANYAA	1914	AAV70486.1
1032	CORYNEBACTERIUM DIPHTHERIAE	SESPNKTVSEEKAKQYLEEF	1915	AAV70486.1
1033	CORYNEBACTERIUM DIPHTHERIAE	SETADNLEKTTAALSILPGI	1916	AAV70486.1
1034	CORYNEBACTERIUM DIPHTHERIAE	SFVMENFSSYHGTKPGYVDS	1917	AAV70486.1
1035	CORYNEBACTERIUM DIPHTHERIAE	SNEISSDSIGVLGYQKTVDH	1918	AAV70486.1
1036	CORYNEBACTERIUM DIPHTHERIAE	SPGHKTQPFLHDGYAVSWNT	1919	AAV70486.1
1037	CORYNEBACTERIUM DIPHTHERIAE	SVNGRKIRMRCRAIDGDVTF	1920	AAV70486.1
1038	CORYNEBACTERIUM DIPHTHERIAE	TAALSILPGIGSVMGIADGA	1921	AAV70486.1
1039	CORYNEBACTERIUM DIPHTHERIAE	TAENTPLPIAGVLLPTIPGK	1922	AAV70486.1
1040	CORYNEBACTERIUM DIPHTHERIAE	TEPLMEQVGTEEFIKRFGDG	1923	AAV70486.1
1041	CORYNEBACTERIUM DIPHTHERIAE	TIKKELGLSLTEPLMEQVGT	1924	AAV70486.1
1042	CORYNEBACTERIUM DIPHTHERIAE	TKVNSKLSLFFEIKS	1925	AAV70486.1
1043	CORYNEBACTERIUM DIPHTHERIAE	VEDSIIRTGFQGESGHDIKI	1926	AAV70486.1
1044	CORYNEBACTERIUM DIPHTHERIAE	VELEINFETRGKRGQDAMYE	1927	AAV70486.1
1045	CORYNEBACTERIUM DIPHTHERIAE	VESIINLFQVVHNSYNRPAY	1928	AAV70486.1
1046	CORYNEBACTERIUM DIPHTHERIAE	VHNSYNRPAYSPGHKTQPFL	1929	AAV70486.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1047	CORYNEBACTERIUM DIPHTHERIAE	VKVTYPGLTKVLALKVDNAE	1930	AAV70486.1
1048	CORYNEBACTERIUM DIPHTHERIAE	VLALKVDNAETIKKELGLSL	1931	AAV70486.1
1049	CORYNEBACTERIUM DIPHTHERIAE	VLGYQKTVDHTKVNSKLSLF	1932	AAV70486.1
1050	CORYNEBACTERIUM DIPHTHERIAE	VRRSVGSSLSCINLDWDVIR	1933	AAV70486.1
1051	CORYNEBACTERIUM DIPHTHERIAE	WAVNVAQVIDSETADNLEKT	1934	AAV70486.1
1052	CORYNEBACTERIUM DIPHTHERIAE	YMAQACAGNRVRRSVGSSLS	1935	AAV70486.1
1053	BORDETELLA PERTUSSIS	AKAPPAPKPAPQPGP	1936	ABO77783.1
1054	BORDETELLA PERTUSSIS	APKPAPQPGP	1937	ABO77783.1
1055	BORDETELLA PERTUSSIS	APKPAPQPGPQPPQP	1938	ABO77783.1
1056	MEASLES VIRUS STRAIN EDMONSTON	AQTRTPLQCTMTEIF	1939	P04851.1
1057	MEASLES VIRUS STRAIN EDMONSTON	ASRGTNMEDEADQYFSHDD	1940	P04851.1
1058	BORDETELLA PERTUSSIS	ATIRR	1941	ABO77783.1
1059	BORDETELLA PERTUSSIS	DNRAG	1942	ABO77783.1
1060	BORDETELLA PERTUSSIS	EAPAPQPPAGRELSA	1943	ABO77783.1
1061	MEASLES VIRUS STRAIN EDMONSTON	EMVRRSAGKVSSTLASELGI	1944	P04851.1
1062	BORDETELLA PERTUSSIS	GASEL	1945	ABO77783.1
1063	BORDETELLA PERTUSSIS	GDALAGGAVP	1946	AAA22980.1
1064	BORDETELLA PERTUSSIS	GDAPAGGAVP	1947	ABO77783.1
1065	BORDETELLA PERTUSSIS	GDTWDDD	1948	ABO77783.1
1066	BORDETELLA PERTUSSIS	GERQH	1949	ABO77783.1
1067	BORDETELLA PERTUSSIS	GGAVP	1950	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1068	BORDETELLA PERTUSSIS	GGFGP	1951	P14283.3
1069	BORDETELLA PERTUSSIS	GGFGPGGFGP	1952	BAF35031.1
1070	BORDETELLA PERTUSSIS	GGFGPVLDGW	1953	ABO77783.1
1071	MEASLES VIRUS STRAIN EDMONSTON	GGKEDRRVKQSRGEARESYR	1954	P04851.1
1072	BORDETELLA PERTUSSIS	GILLEN	1955	ABO77783.1
1073	BORDETELLA PERTUSSIS	GIRRFL	1956	ABO77783.1
1074	MEASLES VIRUS STRAIN EDMONSTON	HDDPISSDQSRFGWFENKEI	1957	P04851.1
1075	MEASLES VIRUS STRAIN EDMONSTON	HEFAGELSTLESLMNLY	1958	P04851.1
1076	BORDETELLA PERTUSSIS	HLGGLAGY	1959	ABO77783.1
1077	MEASLES VIRUS STRAIN EDMONSTON	HTTEDKISRAVGPRQAQVSFL	1960	P04851.1
1078	MEASLES VIRUS STRAIN EDMONSTON	ICDIDTYIVEAGLASFILTI	1961	P04851.1
1079	MEASLES VIRUS STRAIN EDMONSTON	IIIVPIPGDSSITTRSRLLD	1962	P04851.1
1080	MEASLES VIRUS STRAIN EDMONSTON	ILDIKRTPGNKPRIAEMICD	1963	P04851.1
1081	BORDETELLA PERTUSSIS	KALLYR	1964	ABO77783.1
1082	MEASLES VIRUS STRAIN EDMONSTON	KPPITSGSGGAIRGIKHIII	1965	P04851.1
1083	BORDETELLA PERTUSSIS	LAGSGL	1966	ABO77783.1
1084	MEASLES VIRUS STRAIN EDMONSTON	LGITAEDARLVSEIAMHTTE	1967	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1085	MEASLES VIRUS STRAIN EDMONSTON	LGTILAQIWVLLAKAVTA	1968	P04851.1
1086	MEASLES VIRUS STRAIN EDMONSTON	LPTGTPLDIDTASESSQD	1969	P04851.1
1087	MEASLES VIRUS STRAIN EDMONSTON	MATLLRSLALFKRNKDK	1970	P04851.1
1088	BORDETELLA PERTUSSIS	PAPQPP	1971	ABO77783.1
1089	MEASLES VIRUS STRAIN EDMONSTON	PDTAADSELRRWIKYTQQRR	1972	P04851.1
1090	MEASLES VIRUS STRAIN EDMONSTON	PKLTGALIGILSLFVESPGQ	1973	P04851.1
1091	BORDETELLA PERTUSSIS	PQP	1974	ABO77783.1
1092	BORDETELLA PERTUSSIS	PQPGP	1975	ABO77783.1
1093	BORDETELLA PERTUSSIS	PQPGPQPPQPPQPQP	1976	ABO77783.1
1094	MEASLES VIRUS STRAIN EDMONSTON	QDPQDSRRSAEPLLSCKPWQ	1977	P04851.1
1095	MEASLES VIRUS STRAIN EDMONSTON	QRRVVGEFRLERKWLDVVR	1978	P04851.1
1096	BORDETELLA PERTUSSIS	RELSA	1979	ABO77783.1
1097	BORDETELLA PERTUSSIS	RFAPQ	1980	ABO77783.1
1098	MEASLES VIRUS STRAIN EDMONSTON	SIQNKFSAGSYPLLWSYAMG	1981	P04851.1
1099	BORDETELLA PERTUSSIS	SITLQAGAH	1982	ABO77783.1
1100	BORDETELLA PERTUSSIS	SLQPED	1983	ABO77783.1
1101	BORDETELLA PERTUSSIS	SNALSKRL	1984	ABO77783.1
1102	MEASLES VIRUS STRAIN EDMONSTON	SPGQLIQRITDDPDVSIRLL	1985	P04851.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1103	BORDETELLA PERTUSSIS	TELPSIPG	1986	ABO77783.1
1104	BORDETELLA PERTUSSIS	TFTLANK	1987	ABO77783.1
1105	BORDETELLA PERTUSSIS	TWDDD	1988	ABO77783.1
1106	MEASLES VIRUS STRAIN EDMONSTON	VSFLQGDQSENELPRLGGKE	1989	P04851.1
1107	MEASLES VIRUS STRAIN EDMONSTON	WQESRKNKAQTRTPLQC	1990	P04851.1
1108	MEASLES VIRUS STRAIN EDMONSTON	YAMGVGVELENSMGGLNFGR	1991	P04851.1
1109	MEASLES VIRUS STRAIN EDMONSTON	YQQMGKPAPYMVNLENSI	1992	P04851.1
1110	MEASLES VIRUS STRAIN HALLE	MTRSSHQSLVIKLMP	1993	P69355.1
1111	MEASLES VIRUS STRAIN HALLE	PIRDALNAMTQNIRP	1994	P69355.1
1112	RUBELLA VIRUS STRAIN M33	ALLNTPPPYQVSCGGESDRASAGH	1995	CAA28880.1
1113	RUBELLA VIRUS STRAIN THERIEN	GLGSPNCHGPDWASPVCQRHS	1996	P07566.1
1114	RUBELLA VIRUS STRAIN THERIEN	GLGSPNCHGPDWASPVCQRHSPDCSRLV	1997	P07566.1
1115	RUBELLA VIRUS STRAIN THERIEN	NYTGNQQSRWGLGSPNCHGPDWASPV	1998	P07566.1
1116	RUBELLA VIRUS STRAIN THERIEN	TLPQPPCAHGQHYGHHHHQL	1999	P07566.1
1117	RUBELLA VIRUS STRAIN THERIEN	TVRVKFHTETRTVWQLSVAGVSCNVT	2000	P07566.1
1118	RUBELLA VIRUS	ASYFNPGGSYYKQYH	2001	BAA28178.1
1119	RUBELLA VIRUS	FALASYVQHPHKTVR	2002	BAA28178.1
1120	RUBELLA VIRUS	GGESDRASARVIDPAAQSFTG	2003	BAA28178.1
1121	RUBELLA VIRUS	GPGEVWVTPVIGSQARKC	2004	BAA28178.1
1122	RUBELLA VIRUS	GSQARKCGLHIRAG	2005	BAA28178.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1123	RUBELLA VIRUS	LVGATPERPRLRLVDADDPLLRTAP	2006	BAA28178.1
1124	RUBELLA VIRUS	TAPGPGEVWVTPVI	2007	BAA28178.1
1125	MEASLES MORBILLIVIRUS	FIVLSIAYPTLSEIK	2008	AAL29688.1
1126	MEASLES VIRUS STRAIN EDMONSTON-ZAGREB	ETCFQQACKGKIQALCENPEWAPLKDNRIPSY	2009	P08362.1
1127	MEASLES MORBILLIVIRUS	LPRLGGKEDR	2010	P04851.1
1128	MEASLES MORBILLIVIRUS	MSKTEWNASQ	2011	SRC280080
1129	MEASLES MORBILLIVIRUS	SRFGWFENKE	2012	P04851.1
1130	RUBELLA VIRUS	EACVTSWLWSEGEGAVFYRVDLHFINLGT	2013	CAA28880.1
1131	RUBELLA VIRUS	MDFWCVEHDRPPPATPTSLTT	2014	CAA33016.1
1132	RUBELLA VIRUS	PFLGHDGHHGGTLRVGQHHRNASDV	2015	CAA33016.1
1133	RUBELLA VIRUS	RVKFHTETRTVWQLSVAGVSC	2016	BAA19893.1
1134	MEASLES MORBILLIVIRUS	AEEQARHVKNGLE	2017	ABO69699.1
1135	MEASLES MORBILLIVIRUS	ESPQEISKHQALG	2018	SRC280080
1136	MEASLES MORBILLIVIRUS	GVGVELENSMGGLNF	2019	ABI54110.1
1137	MEASLES MORBILLIVIRUS	IKGANDLAKFHQMLMKIIMK	2020	ABO69699.1
1138	MEASLES MORBILLIVIRUS	MSKTLHAQLGFKKT	2021	ABK40528.1
1139	MEASLES MORBILLIVIRUS	NASGLSRPSPSAH	2022	BAE98296.1
1140	MEASLES MORBILLIVIRUS	VRVIDPSLGDRKDE	2023	SRC280148
1141	BORDETELLA PERTUSSIS	DLSDGDLLV	2024	AAC31207.1
1142	BORDETELLA PERTUSSIS	EAERAGRGTG	2025	ACI04548.1
1143	BORDETELLA PERTUSSIS	YRYDSRPPEDV	2026	ACI04548.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1144	BORDETELLA PERTUSSIS	YVDTYGDNAG	2027	ACI04548.1
1145	MEASLES MORBILLIVIRUS	SFSYFYPFR	2028	CAB43772.1
1146	MUMPS RUBULAVIRUS	DIFIVSPR	2029	ADF49557.1
1147	MEASLES MORBILLIVIRUS	QDSRRSADALLRLQAMAGISEEQGS DTDTPIVYNDRN	2030	CAA59302.1
1148	MEASLES MORBILLIVIRUS	SAEALLRLQA	2031	BAH22350.1
1149	MEASLES MORBILLIVIRUS	RIVINREHL	2032	BAB39835.1
1150	MEASLES VIRUS GENOTYPE A	IPRFK	2033	BAB39848.1
1151	BORDETELLA PERTUSSIS	AAALSPMEI	2034	P15318.2
1152	BORDETELLA PERTUSSIS	AAASVVGAPV	2035	P15318.2
1153	BORDETELLA PERTUSSIS	AALGRQDSI	2036	P15318.2
1154	BORDETELLA PERTUSSIS	AAQRLVHAIA	2037	P15318.2
1155	BORDETELLA PERTUSSIS	AAVEAAEL	2038	P15318.2
1156	BORDETELLA PERTUSSIS	AGANVLNGL	2039	P15318.2
1157	BORDETELLA PERTUSSIS	AGYANAAD	2040	P15318.2
1158	BORDETELLA PERTUSSIS	AGYEQFEFRV	2041	P15318.2
1159	BORDETELLA PERTUSSIS	AITGNADNL	2042	P15318.2
1160	BORDETELLA PERTUSSIS	AKEKNATLM	2043	P15318.2
1161	BORDETELLA PERTUSSIS	AKGVFLSL	2044	P15318.2
1162	BORDETELLA PERTUSSIS	APHEYGFGI	2045	P15318.2
1163	BORDETELLA PERTUSSIS	ARQGNDLEI	2046	P15318.2
1164	BORDETELLA PERTUSSIS	ASVVGAPV	2047	P15318.2
1165	BORDETELLA PERTUSSIS	ATLMFRLV	2048	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1166	BORDETELLA PERTUSSIS	AVAAAQRL	2049	P15318.2
1167	BORDETELLA PERTUSSIS	DAGANVLNGL	2050	P15318.2
1168	BORDETELLA PERTUSSIS	DALLAQLYR	2051	P15318.2
1169	BORDETELLA PERTUSSIS	DANGVLKHSI	2052	P15318.2
1170	BORDETELLA PERTUSSIS	DGDMNIGVI	2053	P15318.2
1171	BORDETELLA PERTUSSIS	DHVKNIENL	2054	P15318.2
1172	BORDETELLA PERTUSSIS	DIDMFAIM	2055	P15318.2
1173	BORDETELLA PERTUSSIS	DMFAIMPHL	2056	P15318.2
1174	BORDETELLA PERTUSSIS	DNVRNVENV	2057	P15318.2
1175	BORDETELLA PERTUSSIS	DNVRNVENVI	2058	P15318.2
1176	BORDETELLA PERTUSSIS	DTVDYSAM	2059	P15318.2
1177	BORDETELLA PERTUSSIS	DTVDYSAMI	2060	P15318.2
1178	BORDETELLA PERTUSSIS	DYLRQAGL	2061	P15318.2
1179	BORDETELLA PERTUSSIS	DYYDNVRNV	2062	P15318.2
1180	BORDETELLA PERTUSSIS	EFTTFVEI	2063	P15318.2
1181	BORDETELLA PERTUSSIS	EFTTFVEIV	2064	P15318.2
1182	BORDETELLA PERTUSSIS	EGYVFYEN	2065	P15318.2
1183	BORDETELLA PERTUSSIS	ENVQYRHV	2066	P15318.2
1184	BORDETELLA PERTUSSIS	EQLANSDGL	2067	P15318.2
1185	BORDETELLA PERTUSSIS	FGVGYGHDTI	2068	P15318.2
1186	BORDETELLA PERTUSSIS	FSPDVLETVP	2069	P15318.2
1187	BORDETELLA PERTUSSIS	FSVDHVKNI	2070	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1188	BORDETELLA PERTUSSIS	GDDTYLFGV	2071	P15318.2
1189	BORDETELLA PERTUSSIS	GDDVFLQDL	2072	P15318.2
1190	BORDETELLA PERTUSSIS	GEDGNDIFL	2073	P15318.2
1191	BORDETELLA PERTUSSIS	GERFNVRKQL	2074	P15318.2
1192	BORDETELLA PERTUSSIS	GGAGNDTLV	2075	P15318.2
1193	BORDETELLA PERTUSSIS	GGDDFEAV	2076	P15318.2
1194	BORDETELLA PERTUSSIS	GKSEFTTFV	2077	P15318.2
1195	BORDETELLA PERTUSSIS	GKSLFDDGL	2078	P15318.2
1196	BORDETELLA PERTUSSIS	GNADNLKSV	2079	P15318.2
1197	BORDETELLA PERTUSSIS	GQLVEVDTL	2080	P15318.2
1198	BORDETELLA PERTUSSIS	GRSKFSPDV	2081	P15318.2
1199	BORDETELLA PERTUSSIS	GSSAYDTV	2082	P15318.2
1200	BORDETELLA PERTUSSIS	GTVEKWPAL	2083	P15318.2
1201	BORDETELLA PERTUSSIS	GVDYYDNV	2084	P15318.2
1202	BORDETELLA PERTUSSIS	GYEQFEFRV	2085	P15318.2
1203	BORDETELLA PERTUSSIS	HAVGAQDVV	2086	P15318.2
1204	BORDETELLA PERTUSSIS	IAAGRIGLGI	2087	P15318.2
1205	BORDETELLA PERTUSSIS	IGDAQANTL	2088	P15318.2
1206	BORDETELLA PERTUSSIS	IGLGILADL	2089	P15318.2
1207	BORDETELLA PERTUSSIS	IGNAAGIPL	2090	P15318.2
1208	BORDETELLA PERTUSSIS	IGTSMKDVL	2091	P15318.2
1209	BORDETELLA PERTUSSIS	IGVITDFEL	2092	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1210	BORDETELLA PERTUSSIS	IPLTADIDM	2093	P15318.2
1211	BORDETELLA PERTUSSIS	ISKSALEL	2094	P15318.2
1212	BORDETELLA PERTUSSIS	ITGNADNL	2095	P15318.2
1213	BORDETELLA PERTUSSIS	KIFVVSAT	2096	P15318.2
1214	BORDETELLA PERTUSSIS	KQLNNANVYR	2097	P15318.2
1215	BORDETELLA PERTUSSIS	KVIGNAAGI	2098	P15318.2
1216	BORDETELLA PERTUSSIS	LAKVVSQL	2099	P15318.2
1217	BORDETELLA PERTUSSIS	LANDYARKI	2100	P15318.2
1218	BORDETELLA PERTUSSIS	LDYLRQAGL	2101	P15318.2
1219	BORDETELLA PERTUSSIS	LGKGFASL	2102	P15318.2
1220	BORDETELLA PERTUSSIS	LGKGFASLM	2103	P15318.2
1221	BORDETELLA PERTUSSIS	LGVDYYDN	2104	P15318.2
1222	BORDETELLA PERTUSSIS	LGVDYYDNV	2105	P15318.2
1223	BORDETELLA PERTUSSIS	LKHSIKLDVI	2106	P15318.2
1224	BORDETELLA PERTUSSIS	LQAGYIPV	2107	P15318.2
1225	BORDETELLA PERTUSSIS	LQLTGGTVE	2108	P15318.2
1226	BORDETELLA PERTUSSIS	LSAAVFGL	2109	P15318.2
1227	BORDETELLA PERTUSSIS	LSLGKGFASL	2110	P15318.2
1228	BORDETELLA PERTUSSIS	LSPMEIYGL	2111	P15318.2
1229	BORDETELLA PERTUSSIS	NAHDNFLAGG	2112	P15318.2
1230	BORDETELLA PERTUSSIS	NANVYREGV	2113	P15318.2
1231	BORDETELLA PERTUSSIS	NDTLYGGL	2114	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1232	BORDETELLA PERTUSSIS	NGLAGNDVL	2115	P15318.2
1233	BORDETELLA PERTUSSIS	NNANVYREGV	2116	P15318.2
1234	BORDETELLA PERTUSSIS	NTVSYAAL	2117	P15318.2
1235	BORDETELLA PERTUSSIS	NVLRNIENAV	2118	P15318.2
1236	BORDETELLA PERTUSSIS	PALTFITPL	2119	P15318.2
1237	BORDETELLA PERTUSSIS	PETSNVLRNI	2120	P15318.2
1238	BORDETELLA PERTUSSIS	PMEIYGLV	2121	P15318.2
1239	BORDETELLA PERTUSSIS	PQAYFEKNL	2122	P15318.2
1240	BORDETELLA PERTUSSIS	PVNPNLSKL	2123	P15318.2
1241	BORDETELLA PERTUSSIS	QAGWNASSV	2124	P15318.2
1242	BORDETELLA PERTUSSIS	QAGWNASSVI	2125	P15318.2
1243	BORDETELLA PERTUSSIS	QDAANAGNL	2126	P15318.2
1244	BORDETELLA PERTUSSIS	QDAANAGNLL	2127	P15318.2
1245	BORDETELLA PERTUSSIS	QDSGYDSL	2128	P15318.2
1246	BORDETELLA PERTUSSIS	QQSHYADQL	2129	P15318.2
1247	BORDETELLA PERTUSSIS	RALQGAQAV	2130	P15318.2
1248	BORDETELLA PERTUSSIS	RGGLGLDTL	2131	P15318.2
1249	BORDETELLA PERTUSSIS	RKQLNNANV	2132	P15318.2
1250	BORDETELLA PERTUSSIS	RQDSGYDSL	2133	P15318.2
1251	BORDETELLA PERTUSSIS	RQFRYDGDM	2134	P15318.2
1252	BORDETELLA PERTUSSIS	RSKFSPDVL	2135	P15318.2
1253	BORDETELLA PERTUSSIS	SAGAAAGAL	2136	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1254	BORDETELLA PERTUSSIS	SAHWGQRAL	2137	P15318.2
1255	BORDETELLA PERTUSSIS	SAMIHPGRI	2138	P15318.2
1256	BORDETELLA PERTUSSIS	SAMIHPGRIV	2139	P15318.2
1257	BORDETELLA PERTUSSIS	SAYDTVSGI	2140	P15318.2
1258	BORDETELLA PERTUSSIS	SAYGYEGD	2141	P15318.2
1259	BORDETELLA PERTUSSIS	SGGAGDDVL	2142	P15318.2
1260	BORDETELLA PERTUSSIS	SGLQVAGA	2143	P15318.2
1261	BORDETELLA PERTUSSIS	SGYDSLDGV	2144	P15318.2
1262	BORDETELLA PERTUSSIS	SLLTGALNGI	2145	P15318.2
1263	BORDETELLA PERTUSSIS	SPMEIYGL	2146	P15318.2
1264	BORDETELLA PERTUSSIS	SQMLTRGQL	2147	P15318.2
1265	BORDETELLA PERTUSSIS	SSAYDTVSGI	2148	P15318.2
1266	BORDETELLA PERTUSSIS	SSLAHGHTA	2149	P15318.2
1267	BORDETELLA PERTUSSIS	SSVTSGDSV	2150	P15318.2
1268	BORDETELLA PERTUSSIS	SVIGVQTTEI	2151	P15318.2
1269	BORDETELLA PERTUSSIS	TNTVSYAAL	2152	P15318.2
1270	BORDETELLA PERTUSSIS	TSLIAEGV	2153	P15318.2
1271	BORDETELLA PERTUSSIS	TSLLTGAL	2154	P15318.2
1272	BORDETELLA PERTUSSIS	TVPASPGL	2155	P15318.2
1273	BORDETELLA PERTUSSIS	VAKEKNATL	2156	P15318.2
1274	BORDETELLA PERTUSSIS	VAPHEYGFGI	2157	P15318.2
1275	BORDETELLA PERTUSSIS	VAVVTSLL	2158	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1276	BORDETELLA PERTUSSIS	VFYENRAYG	2159	P15318.2
1277	BORDETELLA PERTUSSIS	VFYENRAYGV	2160	P15318.2
1278	BORDETELLA PERTUSSIS	VITDFELEV	2161	P15318.2
1279	BORDETELLA PERTUSSIS	VNPHSTSL	2162	P15318.2
1280	BORDETELLA PERTUSSIS	VNPHSTSLI	2163	P15318.2
1281	BORDETELLA PERTUSSIS	VNPNLSKL	2164	P15318.2
1282	BORDETELLA PERTUSSIS	VNPNLSKLF	2165	P15318.2
1283	BORDETELLA PERTUSSIS	VQQPIIEKL	2166	P15318.2
1284	BORDETELLA PERTUSSIS	VQYRHVEL	2167	P15318.2
1285	BORDETELLA PERTUSSIS	VSIAAAASV	2168	P15318.2
1286	BORDETELLA PERTUSSIS	VSIAAAASVV	2169	P15318.2
1287	BORDETELLA PERTUSSIS	VTSLLTGAL	2170	P15318.2
1288	BORDETELLA PERTUSSIS	VVLANASRI	2171	P15318.2
1289	BORDETELLA PERTUSSIS	WPALNLFSV	2172	P15318.2
1290	BORDETELLA PERTUSSIS	WVRKASAL	2173	P15318.2
1291	BORDETELLA PERTUSSIS	YAVQYRRKGG	2174	P15318.2
1292	BORDETELLA PERTUSSIS	YGGLGDDTL	2175	P15318.2
1293	BORDETELLA PERTUSSIS	YGLVQQSHYA	2176	P15318.2
1294	BORDETELLA PERTUSSIS	YGYEGDALL	2177	P15318.2
1295	BORDETELLA PERTUSSIS	YIPVNPNL	2178	P15318.2
1296	BORDETELLA PERTUSSIS	YSAMIHPGRI	2179	P15318.2
1297	BORDETELLA PERTUSSIS	YSQTGAHAGI	2180	P15318.2

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1298	CORYNEBACTERIUM DIPHTHERIAE	AYNFVESIINLFQVVHNSYN	2181	CAE11230.1
1299	BORDETELLA PERTUSSIS	SGTTIK	2182	BAF35031.1
1300	BORDETELLA PERTUSSIS	RGHTLESAEGRKIFG	2183	AAA22974.1
1301	BORDETELLA PERTUSSIS	AGAMTVRDVAAAADLALQAGDA	2184	AAA22974.1
1302	BORDETELLA PERTUSSIS	AGAMTVRDVAAAADLALQAGDAL	2185	AAA22974.1
1303	BORDETELLA PERTUSSIS	ALAAVLVNPHIFTRIGAAQTSLADGAAGPA	2186	AAA22974.1
1304	BORDETELLA PERTUSSIS	ALSIDSMTALGA	2187	AAA22974.1
1305	BORDETELLA PERTUSSIS	DLSAARGADISGEGR	2188	AAA22974.1
1306	BORDETELLA PERTUSSIS	DQNRYEYIWGLY	2189	AAA22974.1
1307	BORDETELLA PERTUSSIS	DYTVSADAIALA	2190	AAA22974.1
1308	BORDETELLA PERTUSSIS	GPIVVEAGELVSHAGG	2191	AAA22974.1
1309	BORDETELLA PERTUSSIS	GRPEGLKIGAHSATSVSGSFDAL	2192	AAA22974.1
1310	BORDETELLA PERTUSSIS	ITVTSRGGFDNEGKMESNK	2193	AAA22974.1
1311	BORDETELLA PERTUSSIS	LDQNRYEYIWGLYP	2194	AAA22974.1
1312	BORDETELLA PERTUSSIS	LSAARGADISG	2195	AAA22974.1
1313	BORDETELLA PERTUSSIS	NKIRLMGPLQ	2196	AAA22974.1
1314	BORDETELLA PERTUSSIS	NKLGRIRAGEDM	2197	AAA22974.1
1315	BORDETELLA PERTUSSIS	NKLGRIRAGEDMHLDAPRIE	2198	AAA22974.1
1316	BORDETELLA PERTUSSIS	PHLRNTGQVVAG	2199	AAA22974.1
1317	BORDETELLA PERTUSSIS	QVDLHDLSAARGADISG	2200	AAA22974.1
1318	BORDETELLA PERTUSSIS	RDVAAAADLALQ	2201	AAA22974.1
1319	BORDETELLA PERTUSSIS	SAARGADISGEG	2202	AAA22974.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1320	BORDETELLA PERTUSSIS	TKGEMQIAGKGGGSP	2203	AAA22974.1
1321	BORDETELLA PERTUSSIS	TVSADAIALAAQ	2204	AAA22974.1
1322	BORDETELLA PERTUSSIS	VVAGHDIHI	2205	AAA22974.1
1323	BORDETELLA PERTUSSIS	PSGPNHTKVVQLPKISKNALKANG	2206	CAD12823.1
1324	RUBELLA VIRUS	LVGATPERPRLRLVDADDPLLRTAPG PGEVWVTPVIGSQAR	2207	BAA19902.1
1325	RUBELLA VIRUS	QQSRWGLGSPNCHGPDWASPVCQRH SP	2208	BAA19902.1
1326	BORDETELLA PERTUSSIS	AGEAMVLVYYESIAYSF	2209	ACI04548.1
1327	BORDETELLA PERTUSSIS	GGVGLASTLWYAESNALSKRLGEL	2210	AAZ74322.1
1328	BORDETELLA PERTUSSIS	GTLVRIAPVIGACMARQA	2211	ACI04548.1
1329	BORDETELLA PERTUSSIS	IRRVTRVYHNGITGETTT	2212	ACI04548.1
1330	BORDETELLA PERTUSSIS	IVKTGERQHGIHIQGSDP	2213	AAZ74322.1
1331	BORDETELLA PERTUSSIS	IVKTGERQHGIHIQGSDPGGVRTA	2214	AAZ74338.1
1332	BORDETELLA PERTUSSIS	LRDTNVTAVPASGAPAAVSVLGAS	2215	AAZ74338.1
1333	BORDETELLA PERTUSSIS	PEAPAPQPPAGRELSAAANAAVNT	2216	AAZ74322.1
1334	BORDETELLA PERTUSSIS	AAADFAHAE	2217	WP_019247158.1
1335	BORDETELLA PERTUSSIS	AAAEVAGAL	2218	WP_019249248.1
1336	BORDETELLA PERTUSSIS	AAESTFESY	2219	WP_019247158.1
1337	BORDETELLA PERTUSSIS	AAGFDPEVQ	2220	WP_019248145.1
1338	BORDETELLA PERTUSSIS	AALGRGHSL	2221	AGS56996.1
1339	BORDETELLA PERTUSSIS	AAMQGAVVH	2222	AGT50936.1
1340	BORDETELLA PERTUSSIS	AAPAAHADW	2223	AGS56996.1
1341	BORDETELLA PERTUSSIS	AAQATVVQR	2224	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1342	BORDETELLA PERTUSSIS	AARVAGDNY	2225	WP_019249248.1
1343	BORDETELLA PERTUSSIS	AAVALLNKL	2226	WP_019249248.1
1344	BORDETELLA PERTUSSIS	ADDPPATVY	2227	AAW72734.1
1345	BORDETELLA PERTUSSIS	AEAGRFKVL	2228	AGS56996.1
1346	BORDETELLA PERTUSSIS	AEATQLVTA	2229	WP_019247158.1
1347	BORDETELLA PERTUSSIS	AEGGATLGA	2230	WP_019249248.1
1348	BORDETELLA PERTUSSIS	AEHGEVSIQ	2231	WP_019249248.1
1349	BORDETELLA PERTUSSIS	AEIAFYPKE	2232	WP_019249248.1
1350	BORDETELLA PERTUSSIS	AEKVTTPAV	2233	WP_019247158.1
1351	BORDETELLA PERTUSSIS	AELQTYLRQ	2234	1BCP_C
1352	BORDETELLA PERTUSSIS	AEQSLIEVG	2235	WP_019249248.1
1353	BORDETELLA PERTUSSIS	AESNALSKR	2236	AGS56996.1
1354	BORDETELLA PERTUSSIS	AESSEAMAA	2237	AFK26302.1
1355	BORDETELLA PERTUSSIS	AEVKVGYRA	2238	WP_019247158.1
1356	BORDETELLA PERTUSSIS	AEVTDTSPS	2239	WP_019249248.1
1357	BORDETELLA PERTUSSIS	AGKSLKKKN	2240	WP_019247158.1
1358	BORDETELLA PERTUSSIS	AGLAGPSAV	2241	WP_019249248.1
1359	BORDETELLA PERTUSSIS	AIRVGRGAR	2242	AGT50936.1
1360	BORDETELLA PERTUSSIS	ALAAIASAA	2243	WP_019248145.1
1361	BORDETELLA PERTUSSIS	ALADVPYVL	2244	AAA22983.1
1362	BORDETELLA PERTUSSIS	ALANDGTIV	2245	WP_019248658.1
1363	BORDETELLA PERTUSSIS	ALGRGHSLY	2246	AGS56996.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1364	BORDETELLA PERTUSSIS	ALILAASPV	2247	WP_019248658.1
1365	BORDETELLA PERTUSSIS	ALMLACTGL	2248	AAA22974.1
1366	BORDETELLA PERTUSSIS	AMQGAVVHL	2249	AGT50936.1
1367	BORDETELLA PERTUSSIS	AMTHLSPAL	2250	AAA22983.1
1368	BORDETELLA PERTUSSIS	AMYGKHITL	2251	WP_019249248.1
1369	BORDETELLA PERTUSSIS	ANEANALLW	2252	WP_019249248.1
1370	BORDETELLA PERTUSSIS	APLSITLQA	2253	AGT50936.1
1371	BORDETELLA PERTUSSIS	APNALAWAL	2254	AAA22974.1
1372	BORDETELLA PERTUSSIS	APPAPKPAP	2255	AGS56996.1
1373	BORDETELLA PERTUSSIS	APPGAGFIY	2256	1BCP_C
1374	BORDETELLA PERTUSSIS	APQAAPLSI	2257	AGT50936.1
1375	BORDETELLA PERTUSSIS	APRIENTAK	2258	WP_019249248.1
1376	BORDETELLA PERTUSSIS	AQGKALLYR	2259	AGT50936.1
1377	BORDETELLA PERTUSSIS	AQITSYVGF	2260	WP_019248658.1
1378	BORDETELLA PERTUSSIS	AQLEVRGQR	2261	WP_019249248.1
1379	BORDETELLA PERTUSSIS	AQQLKQADR	2262	WP_019247699.1
1380	BORDETELLA PERTUSSIS	AQVTVAGRY	2263	WP_019249248.1
1381	BORDETELLA PERTUSSIS	ARRSRVRAL	2264	NP_882284.1
1382	BORDETELLA PERTUSSIS	ASPRRARRA	2265	WP_019249248.1
1383	BORDETELLA PERTUSSIS	ASSPDAHVP	2266	AAA22983.1
1384	BORDETELLA PERTUSSIS	ASVSNPGTF	2267	WP_019249248.1
1385	BORDETELLA PERTUSSIS	ATWNFQSTY	2268	WP_019249248.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1386	BORDETELLA PERTUSSIS	ATYIADSGF	2269	AGS56996.1
1387	BORDETELLA PERTUSSIS	AVAAPAVGA	2270	WP_019249248.1
1388	BORDETELLA PERTUSSIS	AVFMQQRPL	2271	AAA22983.1
1389	BORDETELLA PERTUSSIS	AVLVNPHIF	2272	WP_019249248.1
1390	BORDETELLA PERTUSSIS	CFGKDLKRP	2273	AAA22983.1
1391	BORDETELLA PERTUSSIS	CPSSLGNGV	2274	WP_019248145.1
1392	BORDETELLA PERTUSSIS	DAGHEHDTW	2275	AAA22984.1
1393	BORDETELLA PERTUSSIS	DAKHDLTVT	2276	WP_019249248.1
1394	BORDETELLA PERTUSSIS	DASGQHRLW	2277	AGS56996.1
1395	BORDETELLA PERTUSSIS	DATFETYAL	2278	YP_006628018.1
1396	BORDETELLA PERTUSSIS	DATFQTYAL	2279	1BCP_C
1397	BORDETELLA PERTUSSIS	DATLVGAKF	2280	WP_019247158.1
1398	BORDETELLA PERTUSSIS	DDEVDVSGR	2281	AAA22974.1
1399	BORDETELLA PERTUSSIS	DENGKPQTY	2282	WP_019247158.1
1400	BORDETELLA PERTUSSIS	DGPPSRPTT	2283	WP_019247158.1
1401	BORDETELLA PERTUSSIS	DHLTGRSCQ	2284	AAW72734.1
1402	BORDETELLA PERTUSSIS	DNEGKMESN	2285	WP_019249248.1
1403	BORDETELLA PERTUSSIS	DPPATVYRY	2286	AAW72734.1
1404	BORDETELLA PERTUSSIS	EATEGDATL	2287	WP_019247158.1
1405	BORDETELLA PERTUSSIS	EATQQAAGF	2288	WP_019248145.1
1406	BORDETELLA PERTUSSIS	ECSGKQDCP	2289	AAA22983.1
1407	BORDETELLA PERTUSSIS	EGGKLRGKD	2290	AAA22974.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1408	BORDETELLA PERTUSSIS	EGKMESNKD	2291	WP_019249248.1
1409	BORDETELLA PERTUSSIS	EHRMQEAVE	2292	AAW72734.1
1410	BORDETELLA PERTUSSIS	EKRLDIDDA	2293	WP_019249248.1
1411	BORDETELLA PERTUSSIS	EPASANTLL	2294	AGS56996.1
1412	BORDETELLA PERTUSSIS	EPQAELAVF	2295	AGS56996.1
1413	BORDETELLA PERTUSSIS	EPVKLTLTG	2296	AGT50936.1
1414	BORDETELLA PERTUSSIS	ESAEGRKIF	2297	WP_019249248.1
1415	BORDETELLA PERTUSSIS	ESYSESHNF	2298	WP_019247158.1
1416	BORDETELLA PERTUSSIS	ETFCITTIY	2299	1BCP_C
1417	BORDETELLA PERTUSSIS	EVAGALELS	2300	WP_019249248.1
1418	BORDETELLA PERTUSSIS	EVAKVEVVP	2301	WP_019247158.1
1419	BORDETELLA PERTUSSIS	EVDGIIQEF	2302	WP_019249248.1
1420	BORDETELLA PERTUSSIS	EVRADNNFY	2303	WP_019248344.1
1421	BORDETELLA PERTUSSIS	FAILSSTTE	2304	WP_019247158.1
1422	BORDETELLA PERTUSSIS	FAISAYALK	2305	AAA22984.1
1423	BORDETELLA PERTUSSIS	FALYDGTYL	2306	AFK26303.1
1424	BORDETELLA PERTUSSIS	FDTMLGFAI	2307	AAA22984.1
1425	BORDETELLA PERTUSSIS	FEGKPALEL	2308	AAA22983.1
1426	BORDETELLA PERTUSSIS	FELGADHAV	2309	AGS56996.1
1427	BORDETELLA PERTUSSIS	FEPGITTNY	2310	WP_019248658.1
1428	BORDETELLA PERTUSSIS	FETYALTGI	2311	YP_006628018.1
1429	BORDETELLA PERTUSSIS	FIYRETFCI	2312	1BCP_C

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1430	BORDETELLA PERTUSSIS	FPTRTTAPG	2313	NP_882284.1
1431	BORDETELLA PERTUSSIS	FQTYALTGI	2314	1BCP_C
1432	BORDETELLA PERTUSSIS	FTHADGWFL	2315	AGS56996.1
1433	BORDETELLA PERTUSSIS	FVRDGQSVI	2316	1BCP_C
1434	BORDETELLA PERTUSSIS	FVRSGQPVI	2317	YP_006628018.1
1435	BORDETELLA PERTUSSIS	FVWYVDTVI	2318	WP_019248866.1
1436	BORDETELLA PERTUSSIS	GAASSRQAL	2319	WP_019249248.1
1437	BORDETELLA PERTUSSIS	GAASSYFEY	2320	AAW72734.1
1438	BORDETELLA PERTUSSIS	GAFDLKTTF	2321	AFK26303.1
1439	BORDETELLA PERTUSSIS	GAPAAVSVL	2322	AGS56996.1
1440	BORDETELLA PERTUSSIS	GATRAVDSL	2323	AGS56996.1
1441	BORDETELLA PERTUSSIS	GAVPGGAVP	2324	AGT50936.1
1442	BORDETELLA PERTUSSIS	GEAMVLVYY	2325	AFK26302.1
1443	BORDETELLA PERTUSSIS	GEIALGDAT	2326	WP_019249248.1
1444	BORDETELLA PERTUSSIS	GELMAAQVA	2327	WP_019247158.1
1445	BORDETELLA PERTUSSIS	GGVPGGAVP	2328	AAZ74338.1
1446	BORDETELLA PERTUSSIS	GHEHDTWFD	2329	AAA22984.1
1447	BORDETELLA PERTUSSIS	GIGALKAGA	2330	WP_019249248.1
1448	BORDETELLA PERTUSSIS	GIVIPPKAL	2331	1BCP_C
1449	BORDETELLA PERTUSSIS	GKDLKRPGS	2332	AAA22983.1
1450	BORDETELLA PERTUSSIS	GKLPKPVTV	2333	WP_019247158.1
1451	BORDETELLA PERTUSSIS	GKSLKKKNQ	2334	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1452	BORDETELLA PERTUSSIS	GLDVQQGTV	2335	WP_019249248.1
1453	BORDETELLA PERTUSSIS	GLTDGVSRI	2336	WP_019249248.1
1454	BORDETELLA PERTUSSIS	GLYPTYTEW	2337	WP_019249248.1
1455	BORDETELLA PERTUSSIS	GLYQTYTEW	2338	YP_006626470.1
1456	BORDETELLA PERTUSSIS	GPPSRPTTP	2339	WP_019247158.1
1457	BORDETELLA PERTUSSIS	GPSAVAAPA	2340	WP_019249248.1
1458	BORDETELLA PERTUSSIS	GVAPTAQQL	2341	WP_019248866.1
1459	BORDETELLA PERTUSSIS	GVGLASTLW	2342	AGS56996.1
1460	BORDETELLA PERTUSSIS	GYEAGFSLG	2343	WP_019247158.1
1461	BORDETELLA PERTUSSIS	HADDGTIVI	2344	WP_019248145.1
1462	BORDETELLA PERTUSSIS	HADWNNQSI	2345	AGS56996.1
1463	BORDETELLA PERTUSSIS	HAEHEKDVR	2346	WP_019247158.1
1464	BORDETELLA PERTUSSIS	HANHYGTRI	2347	WP_019247158.1
1465	BORDETELLA PERTUSSIS	HAQGKALLY	2348	AGT50936.1
1466	BORDETELLA PERTUSSIS	HFIGYIYEV	2349	AAW72734.1
1467	BORDETELLA PERTUSSIS	HLSPALADV	2350	AAA22983.1
1468	BORDETELLA PERTUSSIS	HSLYASYEY	2351	AGS56996.1
1469	BORDETELLA PERTUSSIS	HVRGMLVPV	2352	AAA22974.1
1470	BORDETELLA PERTUSSIS	HVSKEEQYY	2353	YP_006628018.1
1471	BORDETELLA PERTUSSIS	HVTRGWSIF	2354	AFK26303.1
1472	BORDETELLA PERTUSSIS	IADSGFYLD	2355	AGS56996.1
1473	BORDETELLA PERTUSSIS	IAHRTELRG	2356	AGS56996.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1474	BORDETELLA PERTUSSIS	IENTAKLSG	2357	WP_019249248.1
1475	BORDETELLA PERTUSSIS	IESKISQSV	2358	WP_019249248.1
1476	BORDETELLA PERTUSSIS	IETGGARRF	2359	AGT50936.1
1477	BORDETELLA PERTUSSIS	IIKDAPPGA	2360	1BCP_C
1478	BORDETELLA PERTUSSIS	IIQEFAADL	2361	WP_019249248.1
1479	BORDETELLA PERTUSSIS	ILAGALATY	2362	AAW72734.1
1480	BORDETELLA PERTUSSIS	ILLENPAAE	2363	AGS56996.1
1481	BORDETELLA PERTUSSIS	ILPILVLAL	2364	NP_882286.1
1482	BORDETELLA PERTUSSIS	IPFQRALRL	2365	WP_019248145.1
1483	BORDETELLA PERTUSSIS	ISVRVHVSK	2366	YP_006628018.1
1484	BORDETELLA PERTUSSIS	ITNETGKTY	2367	WP_019247158.1
1485	BORDETELLA PERTUSSIS	ITNKRAALI	2368	WP_019249248.1
1486	BORDETELLA PERTUSSIS	ITSYVGFSV	2369	WP_019248658.1
1487	BORDETELLA PERTUSSIS	ITVTSRGGF	2370	WP_019249248.1
1488	BORDETELLA PERTUSSIS	IVIPPKALF	2371	1BCP_C
1489	BORDETELLA PERTUSSIS	IVVEAGELV	2372	WP_019249248.1
1490	BORDETELLA PERTUSSIS	KAAKSVNLM	2373	WP_019247158.1
1491	BORDETELLA PERTUSSIS	KAAPLRRTT	2374	AGS56996.1
1492	BORDETELLA PERTUSSIS	KAGKLSATG	2375	WP_019249248.1
1493	BORDETELLA PERTUSSIS	KAGTIAAPW	2376	WP_019249248.1
1494	BORDETELLA PERTUSSIS	KAKSLTTEI	2377	WP_019249248.1
1495	BORDETELLA PERTUSSIS	KATVTTVQV	2378	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1496	BORDETELLA PERTUSSIS	KDYRDKDGG	2379	WP_019247158.1
1497	BORDETELLA PERTUSSIS	KEAATIVAA	2380	WP_019249248.1
1498	BORDETELLA PERTUSSIS	KEDVDAAQI	2381	WP_019248658.1
1499	BORDETELLA PERTUSSIS	KEVDGIIQE	2382	WP_019249248.1
1500	BORDETELLA PERTUSSIS	KGPKLAMPW	2383	AGS56996.1
1501	BORDETELLA PERTUSSIS	KLASGGGAV	2384	WP_019249248.1
1502	BORDETELLA PERTUSSIS	KLKGKNQEF	2385	AAA22984.1
1503	BORDETELLA PERTUSSIS	KLLHHILPI	2386	NP_882286.1
1504	BORDETELLA PERTUSSIS	KPAPQPGPQ	2387	AGS56996.1
1505	BORDETELLA PERTUSSIS	KPAPTAPPM	2388	WP_019249248.1
1506	BORDETELLA PERTUSSIS	KPAVSVKVA	2389	WP_019249248.1
1507	BORDETELLA PERTUSSIS	KPDRAARVA	2390	WP_019249248.1
1508	BORDETELLA PERTUSSIS	KPLADIAVI	2391	YP_006626470.1
1509	BORDETELLA PERTUSSIS	KPLADIAVV	2392	WP_019249248.1
1510	BORDETELLA PERTUSSIS	KPLPKPLPV	2393	WP_019247158.1
1511	BORDETELLA PERTUSSIS	KQADRDFVW	2394	WP_019247699.1
1512	BORDETELLA PERTUSSIS	KSLPGGKLP	2395	WP_019247158.1
1513	BORDETELLA PERTUSSIS	KSYTLRYLA	2396	WP_019248658.1
1514	BORDETELLA PERTUSSIS	KTNMVVTSV	2397	AAA22983.1
1515	BORDETELLA PERTUSSIS	KVLAPRLYL	2398	AAA22974.1
1516	BORDETELLA PERTUSSIS	KVLSTKTTL	2399	WP_019247158.1
1517	BORDETELLA PERTUSSIS	LAAGAGLTL	2400	WP_019249248.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1518	BORDETELLA PERTUSSIS	LAAIASAAH	2401	WP_019248145.1
1519	BORDETELLA PERTUSSIS	LAANGNGQW	2402	AGS56996.1
1520	BORDETELLA PERTUSSIS	LAAQVTQRG	2403	WP_019249248.1
1521	BORDETELLA PERTUSSIS	LAARGDGAL	2404	AAA22974.1
1522	BORDETELLA PERTUSSIS	LAAVLVNPH	2405	WP_019249248.1
1523	BORDETELLA PERTUSSIS	LAGSGLFRM	2406	AGS56996.1
1524	BORDETELLA PERTUSSIS	LAKALSAAL	2407	WP_019249248.1
1525	BORDETELLA PERTUSSIS	LALQAGDAL	2408	WP_019249248.1
1526	BORDETELLA PERTUSSIS	LAMPWTFHA	2409	AGS56996.1
1527	BORDETELLA PERTUSSIS	LANDGTIVI	2410	WP_019248658.1
1528	BORDETELLA PERTUSSIS	LAPTVGVAF	2411	WP_019247158.1
1529	BORDETELLA PERTUSSIS	LASDGSVDF	2412	AGS56996.1
1530	BORDETELLA PERTUSSIS	LATYQSEYL	2413	AAW72734.1
1531	BORDETELLA PERTUSSIS	LAWALMLAC	2414	AAA22974.1
1532	BORDETELLA PERTUSSIS	LEAGRRFTH	2415	AGS56996.1
1533	BORDETELLA PERTUSSIS	LFTQQGGAY	2416	1BCP_C
1534	BORDETELLA PERTUSSIS	LKRPGSSPM	2417	AAA22983.1
1535	BORDETELLA PERTUSSIS	LLGSHVARA	2418	AFK26303.1
1536	BORDETELLA PERTUSSIS	LLHHILPIL	2419	NP_882286.1
1537	BORDETELLA PERTUSSIS	LLNAGGTLL	2420	WP_019249248.1
1538	BORDETELLA PERTUSSIS	LMGPLQVNA	2421	WP_019249248.1
1539	BORDETELLA PERTUSSIS	LNDSKITMG	2422	WP_019248145.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1540	BORDETELLA PERTUSSIS	LPEPVKLTL	2423	AGT50936.1
1541	BORDETELLA PERTUSSIS	LPILVLALL	2424	NP_882286.1
1542	BORDETELLA PERTUSSIS	LPKISKNAL	2425	WP_019248145.1
1543	BORDETELLA PERTUSSIS	LPKPVTVKL	2426	WP_019247158.1
1544	BORDETELLA PERTUSSIS	LPLKANPMH	2427	NP_882285.1
1545	BORDETELLA PERTUSSIS	LPPRPVVAE	2428	WP_019247158.1
1546	BORDETELLA PERTUSSIS	LPSIPGTSI	2429	AGS56996.1
1547	BORDETELLA PERTUSSIS	LPTHLYKNF	2430	AAA22984.1
1548	BORDETELLA PERTUSSIS	LPVRGVALV	2431	WP_019249248.1
1549	BORDETELLA PERTUSSIS	LPVSLTALD	2432	WP_019249248.1
1550	BORDETELLA PERTUSSIS	LQGGNKVPV	2433	WP_019249248.1
1551	BORDETELLA PERTUSSIS	LSAALGADW	2434	WP_019249248.1
1552	BORDETELLA PERTUSSIS	LSDAGHEHD	2435	AAA22984.1
1553	BORDETELLA PERTUSSIS	LSGEVQRKG	2436	WP_019249248.1
1554	BORDETELLA PERTUSSIS	LSSPSAITV	2437	WP_019249248.1
1555	BORDETELLA PERTUSSIS	LTWLAILAV	2438	AAW72734.1
1556	BORDETELLA PERTUSSIS	LVFSHVRGM	2439	AAA22974.1
1557	BORDETELLA PERTUSSIS	LVSDAGADL	2440	WP_019249248.1
1558	BORDETELLA PERTUSSIS	LVYYESIAY	2441	AFK26302.1
1559	BORDETELLA PERTUSSIS	MAAESTFES	2442	WP_019247158.1
1560	BORDETELLA PERTUSSIS	MAAGHDATL	2443	WP_019249248.1
1561	BORDETELLA PERTUSSIS	MAAWSERAG	2444	AFK26302.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1562	BORDETELLA PERTUSSIS	MALGALGAA	2445	AGS56996.1
1563	BORDETELLA PERTUSSIS	MAPVMGACM	2446	ADA85123.1
1564	BORDETELLA PERTUSSIS	MATKGEMQI	2447	WP_019249248.1
1565	BORDETELLA PERTUSSIS	MDAKGGTLL	2448	WP_019249248.1
1566	BORDETELLA PERTUSSIS	MESNKDIVI	2449	WP_019249248.1
1567	BORDETELLA PERTUSSIS	MEVMLRAVF	2450	AAA22983.1
1568	BORDETELLA PERTUSSIS	MEYFKTPLP	2451	WP_019249248.1
1569	BORDETELLA PERTUSSIS	MHTIASILL	2452	AAA22984.1
1570	BORDETELLA PERTUSSIS	MIYMSGLAV	2453	1BCP_C
1571	BORDETELLA PERTUSSIS	MLACTGLPL	2454	AAA22974.1
1572	BORDETELLA PERTUSSIS	MPIDRKTLC	2455	AFK26303.1
1573	BORDETELLA PERTUSSIS	MPKAPELDL	2456	WP_019249248.1
1574	BORDETELLA PERTUSSIS	MQRQAGLPL	2457	NP_882285.1
1575	BORDETELLA PERTUSSIS	NALAWALML	2458	AAA22974.1
1576	BORDETELLA PERTUSSIS	NITNKRAAL	2459	WP_019249248.1
1577	BORDETELLA PERTUSSIS	NLMAAESTF	2460	WP_019247158.1
1578	BORDETELLA PERTUSSIS	NNETMSGRQ	2461	WP_019249248.1
1579	BORDETELLA PERTUSSIS	NPGSLIAEV	2462	WP_019249248.1
1580	BORDETELLA PERTUSSIS	NPMHTIASI	2463	NP_882285.1
1581	BORDETELLA PERTUSSIS	NPQTQLSNI	2464	WP_019248145.1
1582	BORDETELLA PERTUSSIS	NPYTSRRSV	2465	AFK26302.1
1583	BORDETELLA PERTUSSIS	PAPTAPPMP	2466	WP_019249248.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1584	BORDETELLA PERTUSSIS	PASANTLLL	2467	AGS56996.1
1585	BORDETELLA PERTUSSIS	PAVALPRPL	2468	WP_019249248.1
1586	BORDETELLA PERTUSSIS	PDAHVPFCF	2469	AAA22983.1
1587	BORDETELLA PERTUSSIS	PELGAAIRV	2470	AGT50936.1
1588	BORDETELLA PERTUSSIS	PFIIKLKDC	2471	WP_019248145.1
1589	BORDETELLA PERTUSSIS	PGPQPPQPP	2472	AGS56996.1
1590	BORDETELLA PERTUSSIS	PGPQPPQPQ	2473	AAZ74338.1
1591	BORDETELLA PERTUSSIS	PGPTTDYST	2474	WP_019248145.1
1592	BORDETELLA PERTUSSIS	PGTFTAGKD	2475	WP_019249248.1
1593	BORDETELLA PERTUSSIS	PGTPGDLLE	2476	AAA22984.1
1594	BORDETELLA PERTUSSIS	PKPKPKAER	2477	WP_019247158.1
1595	BORDETELLA PERTUSSIS	PKPKPKPKA	2478	WP_019247158.1
1596	BORDETELLA PERTUSSIS	PKPKPKPKP	2479	WP_019247158.1
1597	BORDETELLA PERTUSSIS	PLPPRPVVA	2480	WP_019247158.1
1598	BORDETELLA PERTUSSIS	PPAPKPAPQ	2481	AGS56996.1
1599	BORDETELLA PERTUSSIS	PPKPAPVAK	2482	WP_019247158.1
1600	BORDETELLA PERTUSSIS	PPRPVAAQV	2483	WP_019247158.1
1601	BORDETELLA PERTUSSIS	PPRPVVAEK	2484	WP_019247158.1
1602	BORDETELLA PERTUSSIS	PPSRPTTPP	2485	WP_019247158.1
1603	BORDETELLA PERTUSSIS	PRRARRALR	2486	WP_019249248.1
1604	BORDETELLA PERTUSSIS	QAAPLSITL	2487	AGT50936.1
1605	BORDETELLA PERTUSSIS	QADRDFVWY	2488	WP_019247699.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1606	BORDETELLA PERTUSSIS	QAIVVGKDL	2489	WP_019249248.1
1607	BORDETELLA PERTUSSIS	QALGALKLY	2490	ACI16088.1
1608	BORDETELLA PERTUSSIS	QELALKLKG	2491	AAA22984.1
1609	BORDETELLA PERTUSSIS	QITQHGGPY	2492	NP_882283.1
1610	BORDETELLA PERTUSSIS	QITQHGSPY	2493	AFK26303.1
1611	BORDETELLA PERTUSSIS	QPLPPRPVA	2494	WP_019247158.1
1612	BORDETELLA PERTUSSIS	QPPAGRELS	2495	AGS56996.1
1613	BORDETELLA PERTUSSIS	QQLKQADRD	2496	WP_019247699.1
1614	BORDETELLA PERTUSSIS	QQVQVLQRQ	2497	WP_019247158.1
1615	BORDETELLA PERTUSSIS	QSIVEAPEL	2498	AGT50936.1
1616	BORDETELLA PERTUSSIS	QVGSSNSAF	2499	AAW72734.1
1617	BORDETELLA PERTUSSIS	RAGLSPATW	2500	WP_019249248.1
1618	BORDETELLA PERTUSSIS	RARRALRQD	2501	WP_019249248.1
1619	BORDETELLA PERTUSSIS	RASASRARI	2502	WP_019249248.1
1620	BORDETELLA PERTUSSIS	RELSAAANA	2503	AGS56996.1
1621	BORDETELLA PERTUSSIS	RETFCITTI	2504	1BCP_C
1622	BORDETELLA PERTUSSIS	RGFAQRQQL	2505	AGS56996.1
1623	BORDETELLA PERTUSSIS	RGSAATFTL	2506	WP_019248295.1
1624	BORDETELLA PERTUSSIS	RGWSIFALY	2507	AFK26303.1
1625	BORDETELLA PERTUSSIS	RKMLYLIYV	2508	YP_006628018.1
1626	BORDETELLA PERTUSSIS	RLRKMLYLI	2509	YP_006628018.1
1627	BORDETELLA PERTUSSIS	RPQITDAVT	2510	WP_019249248.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1628	BORDETELLA PERTUSSIS	RPSVNGGRI	2511	WP_019249248.1
1629	BORDETELLA PERTUSSIS	RRFTHADGW	2512	AGS56996.1
1630	BORDETELLA PERTUSSIS	RSGARATSL	2513	AAA22974.1
1631	BORDETELLA PERTUSSIS	RSRVRALAW	2514	NP_882284.1
1632	BORDETELLA PERTUSSIS	RSRVRALSW	2515	YP_006628019.1
1633	BORDETELLA PERTUSSIS	RTHGVGASL	2516	AGS56996.1
1634	BORDETELLA PERTUSSIS	RTRGQARSG	2517	AAA22974.1
1635	BORDETELLA PERTUSSIS	RVAPPAVAL	2518	WP_019249248.1
1636	BORDETELLA PERTUSSIS	RVLPEPVKL	2519	AGT50936.1
1637	BORDETELLA PERTUSSIS	RVRALAWLL	2520	NP_882284.1
1638	BORDETELLA PERTUSSIS	RVRALSWLL	2521	YP_006628019.1
1639	BORDETELLA PERTUSSIS	RVTVSGGSL	2522	AGT50936.1
1640	BORDETELLA PERTUSSIS	SEAMAAWSE	2523	AFK26302.1
1641	BORDETELLA PERTUSSIS	SESHNFHAS	2524	WP_019247158.1
1642	BORDETELLA PERTUSSIS	SGEGRVNIG	2525	WP_019249248.1
1643	BORDETELLA PERTUSSIS	SGLAVRVHV	2526	1BCP_C
1644	BORDETELLA PERTUSSIS	SLADISLGA	2527	WP_019249248.1
1645	BORDETELLA PERTUSSIS	SLFAILSST	2528	WP_019247158.1
1646	BORDETELLA PERTUSSIS	SLFAPHGNV	2529	AAZ74338.1
1647	BORDETELLA PERTUSSIS	SLSIDNATW	2530	AGS56996.1
1648	BORDETELLA PERTUSSIS	SPMEVMLRA	2531	AAA22983.1
1649	BORDETELLA PERTUSSIS	SPQPIRATV	2532	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1650	BORDETELLA PERTUSSIS	SPRRARRAL	2533	WP_019249248.1
1651	BORDETELLA PERTUSSIS	SPSRLAGTL	2534	WP_019249248.1
1652	BORDETELLA PERTUSSIS	SSTPLGSLF	2535	WP_019247158.1
1653	BORDETELLA PERTUSSIS	STYELLDYL	2536	WP_019249248.1
1654	BORDETELLA PERTUSSIS	SVAMKPYEV	2537	AAA22983.1
1655	BORDETELLA PERTUSSIS	SVAPNALAW	2538	AAA22974.1
1656	BORDETELLA PERTUSSIS	SVKVAKKLF	2539	WP_019249248.1
1657	BORDETELLA PERTUSSIS	TAFMSGRSL	2540	AAA22984.1
1658	BORDETELLA PERTUSSIS	TAGATPFDI	2541	WP_019248658.1
1659	BORDETELLA PERTUSSIS	TAPVTSPAW	2542	AAW72734.1
1660	BORDETELLA PERTUSSIS	TARTGWLTW	2543	AAW72734.1
1661	BORDETELLA PERTUSSIS	TEAQGVQVR	2544	WP_019248145.1
1662	BORDETELLA PERTUSSIS	TEVYLEHRM	2545	AAW72734.1
1663	BORDETELLA PERTUSSIS	TFEGKPALE	2546	AAA22983.1
1664	BORDETELLA PERTUSSIS	TFTGKVTNG	2547	WP_019248658.1
1665	BORDETELLA PERTUSSIS	TGDGGGHTD	2548	AGS56996.1
1666	BORDETELLA PERTUSSIS	TLAKALSAA	2549	WP_019249248.1
1667	BORDETELLA PERTUSSIS	TLANVGDTW	2550	AGS56996.1
1668	BORDETELLA PERTUSSIS	TLNASNLTL	2551	WP_019249248.1
1669	BORDETELLA PERTUSSIS	TLSSAHGNV	2552	WP_019249248.1
1670	BORDETELLA PERTUSSIS	TPFDIKLKE	2553	WP_019248658.1
1671	BORDETELLA PERTUSSIS	TPFIIKLKD	2554	WP_019248145.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1672	BORDETELLA PERTUSSIS	TPGWSIYGL	2555	1BCP_C
1673	BORDETELLA PERTUSSIS	TPLGSAATF	2556	AGS56996.1
1674	BORDETELLA PERTUSSIS	TPLGSLFAI	2557	WP_019247158.1
1675	BORDETELLA PERTUSSIS	TPLPVSLTA	2558	WP_019249248.1
1676	BORDETELLA PERTUSSIS	TRQGIMDQY	2559	YP_006626873.1
1677	BORDETELLA PERTUSSIS	TSKQDERNY	2560	WP_019247158.1
1678	BORDETELLA PERTUSSIS	TSPYDGKYW	2561	YP_006628018.1
1679	BORDETELLA PERTUSSIS	TSRRSVASI	2562	AFK26302.1
1680	BORDETELLA PERTUSSIS	TSRTVTMRY	2563	NP_879898.1
1681	BORDETELLA PERTUSSIS	TTEYPNARY	2564	ADA85123.1
1682	BORDETELLA PERTUSSIS	TTEYSNARY	2565	AFK26302.1
1683	BORDETELLA PERTUSSIS	TTLGLEQTF	2566	WP_019247158.1
1684	BORDETELLA PERTUSSIS	TVLAAGAGL	2567	WP_019249248.1
1685	BORDETELLA PERTUSSIS	TVQELALKL	2568	AAA22984.1
1686	BORDETELLA PERTUSSIS	TVVQRNKHW	2569	WP_019247158.1
1687	BORDETELLA PERTUSSIS	VAAAADLAL	2570	WP_019249248.1
1688	BORDETELLA PERTUSSIS	VALASQARW	2571	AGS56996.1
1689	BORDETELLA PERTUSSIS	VAMKPYEVT	2572	AAA22983.1
1690	BORDETELLA PERTUSSIS	VARLVKLQG	2573	WP_019249248.1
1691	BORDETELLA PERTUSSIS	VAVAGGRWH	2574	AGS56996.1
1692	BORDETELLA PERTUSSIS	VEASAITTY	2575	WP_019248145.1
1693	BORDETELLA PERTUSSIS	VEDIGGKNY	2576	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1694	BORDETELLA PERTUSSIS	VEVSSPPPV	2577	WP_019247158.1
1695	BORDETELLA PERTUSSIS	VGAGGYEAG	2578	WP_019247158.1
1696	BORDETELLA PERTUSSIS	VGGGEHGRW	2579	WP_019249248.1
1697	BORDETELLA PERTUSSIS	VHVSKEEQY	2580	YP_006628018.1
1698	BORDETELLA PERTUSSIS	VIDGQKVLA	2581	AAA22974.1
1699	BORDETELLA PERTUSSIS	VIGACTSPY	2582	YP_006628018.1
1700	BORDETELLA PERTUSSIS	VKLGGVYEA	2583	AAA22974.1
1701	BORDETELLA PERTUSSIS	VLAPRLYLT	2584	AAA22974.1
1702	BORDETELLA PERTUSSIS	VLVKTNMVV	2585	AAA22983.1
1703	BORDETELLA PERTUSSIS	VPASGAPAA	2586	AGS56996.1
1704	BORDETELLA PERTUSSIS	VPFCFGKDL	2587	AAA22983.1
1705	BORDETELLA PERTUSSIS	VPVSEHCTV	2588	AAA22974.1
1706	BORDETELLA PERTUSSIS	VPVTPPKVE	2589	WP_019247158.1
1707	BORDETELLA PERTUSSIS	VRTVSAMEY	2590	WP_019249248.1
1708	BORDETELLA PERTUSSIS	VSGGSLSAP	2591	AGT50936.1
1709	BORDETELLA PERTUSSIS	VSSATKAKG	2592	WP_019248658.1
1710	BORDETELLA PERTUSSIS	VSSPPPVSV	2593	WP_019247158.1
1711	BORDETELLA PERTUSSIS	VSVKVAKKL	2594	WP_019249248.1
1712	BORDETELLA PERTUSSIS	VTMRYLASY	2595	WP_019248145.1
1713	BORDETELLA PERTUSSIS	VTSVAMKPY	2596	AAA22983.1
1714	BORDETELLA PERTUSSIS	VVAEKVTTP	2597	WP_019247158.1
1715	BORDETELLA PERTUSSIS	VVDGPPSRP	2598	WP_019247158.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1716	BORDETELLA PERTUSSIS	VVETAQPLP	2599	WP_019247158.1
1717	BORDETELLA PERTUSSIS	WLTWLAILA	2600	AAW72734.1
1718	BORDETELLA PERTUSSIS	WTFHAGYRY	2601	AGS56996.1
1719	BORDETELLA PERTUSSIS	WVMTDNSNV	2602	AGS56996.1
1720	BORDETELLA PERTUSSIS	YAEHGEVSI	2603	WP_019249248.1
1721	BORDETELLA PERTUSSIS	YAIDGTAAG	2604	WP_019249248.1
1722	BORDETELLA PERTUSSIS	YALKSRIAL	2605	AAA22984.1
1723	BORDETELLA PERTUSSIS	YATNPQTQL	2606	WP_019248145.1
1724	BORDETELLA PERTUSSIS	YDTGDLIAY	2607	WP_019248658.1
1725	BORDETELLA PERTUSSIS	YEAGFSLGS	2608	WP_019247158.1
1726	BORDETELLA PERTUSSIS	YEDATFETY	2609	YP_006628018.1
1727	BORDETELLA PERTUSSIS	YENKSSTPL	2610	WP_019247158.1
1728	BORDETELLA PERTUSSIS	YEVTPTRML	2611	AAA22983.1
1729	BORDETELLA PERTUSSIS	YEYIWGLYP	2612	WP_019249248.1
1730	BORDETELLA PERTUSSIS	YEYIWGLYQ	2613	YP_006626470.1
1731	BORDETELLA PERTUSSIS	YEYSKGPKL	2614	AGS56996.1
1732	BORDETELLA PERTUSSIS	YFEPGPTTD	2615	WP_019248145.1
1733	BORDETELLA PERTUSSIS	YIWGLYPTY	2616	WP_019249248.1
1734	BORDETELLA PERTUSSIS	YIWGLYQTY	2617	YP_006626470.1
1735	BORDETELLA PERTUSSIS	YLRQITPGW	2618	1BCP_C
1736	BORDETELLA PERTUSSIS	YMIYMSGLA	2619	1BCP_C
1737	BORDETELLA PERTUSSIS	YPALRAALI	2620	WP_019248658.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1738	BORDETELLA PERTUSSIS	YPGTPGDLL	2621	AAA22984.1
1739	BORDETELLA PERTUSSIS	YPTYTEWSV	2622	WP_019249248.1
1740	BORDETELLA PERTUSSIS	YQTYTEWSV	2623	YP_006626470.1
1741	BORDETELLA PERTUSSIS	YSTGDLRAY	2624	WP_019248145.1
1742	BORDETELLA PERTUSSIS	YTLRYLASY	2625	WP_019248658.1
1743	BORDETELLA PERTUSSIS	YVLVKTNMV	2626	AAA22983.1
1744	BORDETELLA PERTUSSIS	YYDYEDATF	2627	YP_006628018.1
1745	BORDETELLA PERTUSSIS 509	AAFIALYPNSQLAPT	2628	Q7VU05
1746	BORDETELLA PERTUSSIS 509	GGAEYNLALGQRRA	2629	Q7VU04
1747	BORDETELLA PERTUSSIS 509	GGAEYNLALGQRRADA	2630	Q7VU04
1748	BORDETELLA PERTUSSIS 509	IALYPNSQLAPT	2631	Q7VU05
1749	BORDETELLA PERTUSSIS 509	KPDQGEVVAVGPGKKTED	2632	P0A339.1
1750	BORDETELLA PERTUSSIS 509	KPDQGEVVAVGPGKKTEDG	2633	P0A339.1
1751	BORDETELLA PERTUSSIS 509	LAEVLDYHNFVLTQ	2634	Q7VWM1.1
1752	CORYNEBACTERIUM DIPHTHERIAE	QSIALSSLMVAQAIP	2635	AAV70486.1
1753	CORYNEBACTERIUM DIPHTHERIAE	SIGVLGYQKTVDHTKVNSKLSLF	2636	AAV70486.1
1754	BORDETELLA PERTUSSIS	AAHADWNNQSIVKT	2637	ABO77783.1
1755	BORDETELLA PERTUSSIS	AALGRG	2638	ABO77783.1
1756	BORDETELLA PERTUSSIS	AALGRGHSLYASYE	2639	ABO77783.1
1757	BORDETELLA PERTUSSIS	AAPLRRTTLAMALG	2640	ABO77783.1
1758	BORDETELLA PERTUSSIS	AAPLSITLQAGAHA	2641	ABO77783.1
1759	BORDETELLA PERTUSSIS	ADAQGDIVATELPS	2642	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1760	BORDETELLA PERTUSSIS	ADSGFYLDATLRAS	2643	ABO77783.1
1761	BORDETELLA PERTUSSIS	AELA	2644	ABO77783.1
1762	BORDETELLA PERTUSSIS	AELAVFRAGGGAYR	2645	ABO77783.1
1763	BORDETELLA PERTUSSIS	AELQFRNGSVTSSG	2646	ABO77783.1
1764	BORDETELLA PERTUSSIS	AGGRWHLGGLAGYT	2647	ABO77783.1
1765	BORDETELLA PERTUSSIS	AGVAAMQGAVVHLQ	2648	ABO77783.1
1766	BORDETELLA PERTUSSIS	AGYTRGDRGFTGDG	2649	ABO77783.1
1767	BORDETELLA PERTUSSIS	ALASQARWTGATRA	2650	ABO77783.1
1768	BORDETELLA PERTUSSIS	AMPWTFHAGYRYSW	2651	ABO77783.1
1769	BORDETELLA PERTUSSIS	AMQGAVVHLQRATIRRGDAP	2652	ABO77783.1
1770	BORDETELLA PERTUSSIS	ANGLRVRDE	2653	ABO77783.1
1771	BORDETELLA PERTUSSIS	ANGLRVRDEGGSSV	2654	ABO77783.1
1772	BORDETELLA PERTUSSIS	ANKDGKVDIGTYRY	2655	ABO77783.1
1773	BORDETELLA PERTUSSIS	APAAVSVLGASELT	2656	ABO77783.1
1774	BORDETELLA PERTUSSIS	APPAPKPAPQPGPQ	2657	ABO77783.1
1775	BORDETELLA PERTUSSIS	AQGILLENPAAELQ	2658	ABO77783.1
1776	BORDETELLA PERTUSSIS	ARWTGATRAVDSLS	2659	ABO77783.1
1777	BORDETELLA PERTUSSIS	ASLEAGRRFTHADG	2660	ABO77783.1
1778	BORDETELLA PERTUSSIS	ASYEYSKGPKLAMP	2661	ABO77783.1
1779	BORDETELLA PERTUSSIS	ATFTLANKD	2662	ABO77783.1
1780	BORDETELLA PERTUSSIS	ATFTLANKDGKVDI	2663	ABO77783.1
1781	BORDETELLA PERTUSSIS	ATRAVDSLSIDNAT	2664	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1782	BORDETELLA PERTUSSIS	DDDGIALYVAGEQAQ	2665	ABO77783.1
1783	BORDETELLA PERTUSSIS	DDGIALYVAGEQAQ	2666	ABO77783.1
1784	BORDETELLA PERTUSSIS	DGGHITGGRAAGVA	2667	ABO77783.1
1785	BORDETELLA PERTUSSIS	DGIRRFLGTVTVKAGK	2668	ABO77783.1
1786	BORDETELLA PERTUSSIS	DGSVDFQQPAEAGR	2669	ABO77783.1
1787	BORDETELLA PERTUSSIS	DGYAVKGKYRTHGV	2670	ABO77783.1
1788	BORDETELLA PERTUSSIS	DIVATELPSIPGTS	2671	ABO77783.1
1789	BORDETELLA PERTUSSIS	DKLVVMQDASGQHR	2672	ABO77783.1
1790	BORDETELLA PERTUSSIS	DLGLSDKLVVMQDA	2673	ABO77783.1
1791	BORDETELLA PERTUSSIS	DNATWVMTDNSNVGA	2674	ABO77783.1
1792	BORDETELLA PERTUSSIS	DNATWVMTDNSNVGALRLA	2675	ABO77783.1
1793	BORDETELLA PERTUSSIS	DNRAGRRFDQKVAG	2676	ABO77783.1
1794	BORDETELLA PERTUSSIS	EAGRFKVLTVNTLA	2677	ABO77783.1
1795	BORDETELLA PERTUSSIS	ELAQSIVEAPELGA	2678	ABO77783.1
1796	BORDETELLA PERTUSSIS	ELGAAIRVGRGARV	2679	ABO77783.1
1797	BORDETELLA PERTUSSIS	ELGADHAVAVAGGR	2680	ABO77783.1
1798	BORDETELLA PERTUSSIS	ELPSIPGTSIGPLD	2681	ABO77783.1
1799	BORDETELLA PERTUSSIS	EPVKLTLTGGADAQ	2682	ABO77783.1
1800	BORDETELLA PERTUSSIS	EQAQASIADSTLQG	2683	ABO77783.1
1801	BORDETELLA PERTUSSIS	ERGANVTVQRSAIV	2684	ABO77783.1
1802	BORDETELLA PERTUSSIS	ERQHGIHIQGSDPG	2685	ABO77783.1
1803	BORDETELLA PERTUSSIS	EVGKRIELAGGRQV	2686	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1804	BORDETELLA PERTUSSIS	FDGAGTVHTNGIAH	2687	ABO77783.1
1805	BORDETELLA PERTUSSIS	FQQPAEAGRFKVLT	2688	ABO77783.1
1806	BORDETELLA PERTUSSIS	FRAGGGAYRAANGL	2689	ABO77783.1
1807	BORDETELLA PERTUSSIS	GAHAQGKALLYRVL	2690	ABO77783.1
1808	BORDETELLA PERTUSSIS	GARVTVSGGSLSAP	2691	ABO77783.1
1809	BORDETELLA PERTUSSIS	GAYRAANGLRVRDE	2692	ABO77783.1
1810	BORDETELLA PERTUSSIS	GDAPAGGAVPGGAV	2693	ABO77783.1
1811	BORDETELLA PERTUSSIS	GGAVPGGAVPGGFG	2694	ABO77783.1
1812	BORDETELLA PERTUSSIS	GGAVPGGFGPVLDG	2695	ABO77783.1
1813	BORDETELLA PERTUSSIS	GGFGPVLDGWYGVD	2696	ABO77783.1
1814	BORDETELLA PERTUSSIS	GGLHIGALQSLQPE	2697	ABO77783.1
1815	BORDETELLA PERTUSSIS	GGVQIERGANVTVQ	2698	ABO77783.1
1816	BORDETELLA PERTUSSIS	GHSLYASYEYSKGP	2699	ABO77783.1
1817	BORDETELLA PERTUSSIS	GHTDSVHVGGYATY	2700	ABO77783.1
1818	BORDETELLA PERTUSSIS	GIAHRTELRGTRAE	2701	ABO77783.1
1819	BORDETELLA PERTUSSIS	GKALLYRVLPEPVK	2702	ABO77783.1
1820	BORDETELLA PERTUSSIS	GLGMAAALGRGHSL	2703	ABO77783.1
1821	BORDETELLA PERTUSSIS	GNVIETGGARRFAP	2704	ABO77783.1
1822	BORDETELLA PERTUSSIS	GPLDVALASQARWT	2705	ABO77783.1
1823	BORDETELLA PERTUSSIS	GQHRLWVRN	2706	ABO77783.1
1824	BORDETELLA PERTUSSIS	GRGFAQRQQLDNRA	2707	ABO77783.1
1825	BORDETELLA PERTUSSIS	GRLGLEVGKRIELA	2708	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1826	BORDETELLA PERTUSSIS	GRQVQPYIKASVLQ	2709	ABO77783.1
1827	BORDETELLA PERTUSSIS	GRRFTHADGWFLEPQAELA	2710	ABO77783.1
1828	BORDETELLA PERTUSSIS	GSEPASANTLLLVQ	2711	ABO77783.1
1829	BORDETELLA PERTUSSIS	GSSVLGRLGLEVGK	2712	ABO77783.1
1830	BORDETELLA PERTUSSIS	GTTIKVSGRQAQGI	2713	ABO77783.1
1831	BORDETELLA PERTUSSIS	GTVTVKAGKLVADH	2714	ABO77783.1
1832	BORDETELLA PERTUSSIS	HAVAVAGGRWHLGG	2715	ABO77783.1
1833	BORDETELLA PERTUSSIS	IELAGGRQVQPYIK	2716	ABO77783.1
1834	BORDETELLA PERTUSSIS	IHIQGSDPGGVRTA	2717	ABO77783.1
1835	BORDETELLA PERTUSSIS	IRRFLGTVTVKAGK	2718	ABO77783.1
1836	BORDETELLA PERTUSSIS	IRVGRGARVTVSGG	2719	ABO77783.1
1837	BORDETELLA PERTUSSIS	ITLQAGAHA	2720	ABO77783.1
1838	BORDETELLA PERTUSSIS	ITLQAGAHAQGKAL	2721	ABO77783.1
1839	BORDETELLA PERTUSSIS	IVEAPELGAAIRVG	2722	ABO77783.1
1840	BORDETELLA PERTUSSIS	IVKTGERQHGIHIQ	2723	ABO77783.1
1841	BORDETELLA PERTUSSIS	KAGKLVADHATLAN	2724	ABO77783.1
1842	BORDETELLA PERTUSSIS	KGKYRTHGVGASLE	2725	ABO77783.1
1843	BORDETELLA PERTUSSIS	KPAPQPGPQPPQPP	2726	ABO77783.1
1844	BORDETELLA PERTUSSIS	KVAGFELGADHAVA	2727	ABO77783.1
1845	BORDETELLA PERTUSSIS	KVAGSDGYAVKGKY	2728	ABO77783.1
1846	BORDETELLA PERTUSSIS	KVDIGTYRYRLAAN	2729	ABO77783.1
1847	BORDETELLA PERTUSSIS	KVLTVNTLAGSGLF	2730	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1848	BORDETELLA PERTUSSIS	LAANGNGQWSLVGA	2731	ABO77783.1
1849	BORDETELLA PERTUSSIS	LAMPWTFHAGYRYS	2732	ABO77783.1
1850	BORDETELLA PERTUSSIS	LASTLWYAESNALS	2733	ABO77783.1
1851	BORDETELLA PERTUSSIS	LENDFKVAGSDGYA	2734	ABO77783.1
1852	BORDETELLA PERTUSSIS	LENPAAELQFRNGS	2735	ABO77783.1
1853	BORDETELLA PERTUSSIS	LGAAPAAHADWNNQ	2736	ABO77783.1
1854	BORDETELLA PERTUSSIS	LGGLAGYTRGDRGFTGDG	2737	ABO77783.1
1855	BORDETELLA PERTUSSIS	LLENP	2738	ABO77783.1
1856	BORDETELLA PERTUSSIS	LLVQTPLGSAATFT	2739	ABO77783.1
1857	BORDETELLA PERTUSSIS	LPPSRVVLRDTNVT	2740	ABO77783.1
1858	BORDETELLA PERTUSSIS	LQPEDLPPS	2741	ABO77783.1
1859	BORDETELLA PERTUSSIS	LQPEDLPPSRVVLR	2742	ABO77783.1
1860	BORDETELLA PERTUSSIS	LRASRLENDFKVAG	2743	ABO77783.1
1861	BORDETELLA PERTUSSIS	LRLASDGSVDFQQP	2744	ABO77783.1
1862	BORDETELLA PERTUSSIS	LSAAANAAVNTGGV	2745	ABO77783.1
1863	BORDETELLA PERTUSSIS	LSAPHGNVIETGGA	2746	ABO77783.1
1864	BORDETELLA PERTUSSIS	LSDDGIRRFLGTVT	2747	ABO77783.1
1865	BORDETELLA PERTUSSIS	LVGAKAPPAPKPAP	2748	ABO77783.1
1866	BORDETELLA PERTUSSIS	LYVAGEQAQASIAD	2749	ABO77783.1
1867	BORDETELLA PERTUSSIS	MALGALGAAPAAHA	2750	ABO77783.1
1868	BORDETELLA PERTUSSIS	MNMSLSRIVKAAPL	2751	ABO77783.1
1869	BORDETELLA PERTUSSIS	MQDASGQHR	2752	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1870	BORDETELLA PERTUSSIS	MQGAVVHLQRATIR	2753	ABO77783.1
1871	BORDETELLA PERTUSSIS	NAAVNTGGVGLAST	2754	ABO77783.1
1872	BORDETELLA PERTUSSIS	NALSKRLGELRLNP	2755	ABO77783.1
1873	BORDETELLA PERTUSSIS	NGQWSLVGAKAPPA	2756	ABO77783.1
1874	BORDETELLA PERTUSSIS	NTLAGSGLFRMNVF	2757	ABO77783.1
1875	BORDETELLA PERTUSSIS	PAGRELSAAANAAV	2758	ABO77783.1
1876	BORDETELLA PERTUSSIS	PAPQPPAGRELSAA	2759	ABO77783.1
1877	BORDETELLA PERTUSSIS	PGPQPPQPPQPQPE	2760	ABO77783.1
1878	BORDETELLA PERTUSSIS	PGTSIGPLDVALAS	2761	ABO77783.1
1879	BORDETELLA PERTUSSIS	PLGSAATFTLANKD	2762	ABO77783.1
1880	BORDETELLA PERTUSSIS	PQPEAPAPQPPAGR	2763	ABO77783.1
1881	BORDETELLA PERTUSSIS	PQPPQPQPEAPAPQ	2764	ABO77783.1
1882	BORDETELLA PERTUSSIS	PYIKASVLQEFDGA	2765	ABO77783.1
1883	BORDETELLA PERTUSSIS	RFAPQAAPLSITLQ	2766	ABO77783.1
1884	BORDETELLA PERTUSSIS	RLGELRLNPDAGGA	2767	ABO77783.1
1885	BORDETELLA PERTUSSIS	RLNPDAGGAWGRGF	2768	ABO77783.1
1886	BORDETELLA PERTUSSIS	RNGSVTSSGQLSDD	2769	ABO77783.1
1887	BORDETELLA PERTUSSIS	RRFDQKVAGFELGA	2770	ABO77783.1
1888	BORDETELLA PERTUSSIS	RTTLAMALGALGAA	2771	ABO77783.1
1889	BORDETELLA PERTUSSIS	SAIVDGGLHIGALQ	2772	ABO77783.1
1890	BORDETELLA PERTUSSIS	SANTLLLVQTPLGS	2773	ABO77783.1
1891	BORDETELLA PERTUSSIS	SDPGGVRTASGTTI	2774	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1892	BORDETELLA PERTUSSIS	SELTLDGGHITGGR	2775	ABO77783.1
1893	BORDETELLA PERTUSSIS	SGLFRMNVF	2776	ABO77783.1
1894	BORDETELLA PERTUSSIS	SGLFRMNVFADLGL	2777	ABO77783.1
1895	BORDETELLA PERTUSSIS	SGSSVELAQSIVEA	2778	ABO77783.1
1896	BORDETELLA PERTUSSIS	SIADSTLQGAGGVQ	2779	ABO77783.1
1897	BORDETELLA PERTUSSIS	SKGPKLAMPWTFHA	2780	ABO77783.1
1898	BORDETELLA PERTUSSIS	SNVGALRLASDGSV	2781	ABO77783.1
1899	BORDETELLA PERTUSSIS	SRIVKAAPLRRTTL	2782	ABO77783.1
1900	BORDETELLA PERTUSSIS	SVLGASELTLDGGH	2783	ABO77783.1
1901	BORDETELLA PERTUSSIS	SVLQEFDGA	2784	ABO77783.1
1902	BORDETELLA PERTUSSIS	SVLQEFDGAGTVHT	2785	ABO77783.1
1903	BORDETELLA PERTUSSIS	TELR	2786	ABO77783.1
1904	BORDETELLA PERTUSSIS	TELRGTRAELGLGM	2787	ABO77783.1
1905	BORDETELLA PERTUSSIS	TGDGGGHTDSVHVG	2788	ABO77783.1
1906	BORDETELLA PERTUSSIS	TGGARRFAPQAAPL	2789	ABO77783.1
1907	BORDETELLA PERTUSSIS	TGGRAAGVAAMQGA	2790	ABO77783.1
1908	BORDETELLA PERTUSSIS	TGGVGLASTLWYAE	2791	ABO77783.1
1909	BORDETELLA PERTUSSIS	THGVGASLEAGRRF	2792	ABO77783.1
1910	BORDETELLA PERTUSSIS	TIRRGDAPA	2793	ABO77783.1
1911	BORDETELLA PERTUSSIS	TLANVGDTWDDDGI	2794	ABO77783.1
1912	BORDETELLA PERTUSSIS	TLQGAGGVQIERGA	2795	ABO77783.1
1913	BORDETELLA PERTUSSIS	TLTGGADAQGDIVA	2796	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1914	BORDETELLA PERTUSSIS	TNVTAVPASGAPAA	2797	ABO77783.1
1915	BORDETELLA PERTUSSIS	TRAELGLGMAAALG	2798	ABO77783.1
1916	BORDETELLA PERTUSSIS	TSSGQLSDDGIRRF	2799	ABO77783.1
1917	BORDETELLA PERTUSSIS	TVHTNGIAHRTELR	2800	ABO77783.1
1918	BORDETELLA PERTUSSIS	TYRYRLAANGNGQW	2801	ABO77783.1
1919	BORDETELLA PERTUSSIS	VADHATLANVGDTW	2802	ABO77783.1
1920	BORDETELLA PERTUSSIS	VHVGGYATYIADSG	2803	ABO77783.1
1921	BORDETELLA PERTUSSIS	VLDGWYGVD	2804	ABO77783.1
1922	BORDETELLA PERTUSSIS	VPASGAPAAVSVLG	2805	ABO77783.1
1923	BORDETELLA PERTUSSIS	VRDEGGSSVLGRLG	2806	ABO77783.1
1924	BORDETELLA PERTUSSIS	VRTASGTTIKVSGR	2807	ABO77783.1
1925	BORDETELLA PERTUSSIS	VSGGSLSAPHGNVI	2808	ABO77783.1
1926	BORDETELLA PERTUSSIS	VSGRQAQGILLENP	2809	ABO77783.1
1927	BORDETELLA PERTUSSIS	VTVQRSAIVDGGLH	2810	ABO77783.1
1928	BORDETELLA PERTUSSIS	VVLRDTNVTAVPAS	2811	ABO77783.1
1929	BORDETELLA PERTUSSIS	WNNQSIVKTGERQH	2812	ABO77783.1
1930	BORDETELLA PERTUSSIS	WVRNSGSEPASANT	2813	ABO77783.1
1931	BORDETELLA PERTUSSIS	WYAESNALSKRLGE	2814	ABO77783.1
1932	BORDETELLA PERTUSSIS	YATYIADSGFYLDA	2815	ABO77783.1
1933	BORDETELLA PERTUSSIS	YGVDVSGSS	2816	ABO77783.1
1934	BORDETELLA PERTUSSIS	YGVDVSGSSVELAQ	2817	ABO77783.1
1935	BORDETELLA PERTUSSIS	YLDATLRASRLEND	2818	ABO77783.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1936	BORDETELLA PERTUSSIS	YRVLPEPVKLTLTG	2819	ABO77783.1
1937	BORDETELLA PERTUSSIS	VKAQNITNKRAALIEA	2820	AAA22974.1
1938	BORDETELLA PERTUSSIS	YYSNVTATRLLSSTNS	2821	AAA83981.1
1939	BORDETELLA PERTUSSIS	SPNLTDERAAQAGVT	2822	CPP72976.1
1940	MEASLES MORBILLIVIRUS	SSRASDERAAHLPTS	2823	BAA33867.1
1941	CORYNEBACTERIUM DIPHTHERIAE	QVVHNSYNRPAYSPG	2824	1007216A
1942	MEASLES VIRUS STRAIN EDMONSTON-B	AEGGEIHEL	2825	AAF85692.1
1943	MEASLES VIRUS STRAIN EDMONSTON-B	AENLISNGIGKY	2826	AAF85698.1
1944	MEASLES VIRUS STRAIN EDMONSTON-B	AEVDGDVKL	2827	CAB43772.1
1945	MEASLES VIRUS STRAIN EDMONSTON-B	AIYTAEIHK	2828	AAF85697.1
1946	MEASLES VIRUS STRAIN EDMONSTON-B	APVFHMTNY	2829	CAB43772.1
1947	MEASLES VIRUS STRAIN EDMONSTON-B	APVFHMTNYLEQPVSN	2830	AAR89413.1
1948	MEASLES VIRUS STRAIN EDMONSTON-B	AQRLNEIY	2831	AAF85698.1
1949	MEASLES VIRUS STRAIN EDMONSTON-B	ARVPHAYSL	2832	AAF85698.1
1950	MEASLES VIRUS STRAIN EDMONSTON-B	AVRDLERAM	2833	P03424.1
1951	MEASLES VIRUS STRAIN EDMONSTON-B	AVRDLERAMTTLK	2834	P03424.1
1952	MEASLES VIRUS STRAIN EDMONSTON-B	DALLRLQAM	2835	Q89933.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1953	MEASLES VIRUS STRAIN EDMONSTON-B	DIKEKVINL	2836	AAF85698.1
1954	MEASLES VIRUS STRAIN EDMONSTON-B	DQGLFKVL	2837	AAF85695.1
1955	MEASLES VIRUS STRAIN EDMONSTON-B	DTGVDTRIW	2838	Q9EMA9.1
1956	MEASLES VIRUS STRAIN EDMONSTON-B	EPIGSLAIEEAM	2839	AAF85692.1
1957	MEASLES VIRUS STRAIN EDMONSTON-B	EPIRDALNAM	2840	P69354.1
1958	MEASLES VIRUS STRAIN EDMONSTON-B	FPKLGKTL	2841	AAF85692.1
1959	MEASLES VIRUS STRAIN EDMONSTON-B	FRSVNAVAF	2842	AAF85695.1
1960	MEASLES VIRUS STRAIN EDMONSTON-B	GKIIDNTEQL	2843	AAF85695.1
1961	MEASLES VIRUS STRAIN EDMONSTON-B	GLNEKLVFY	2844	AAF85695.1
1962	MEASLES VIRUS STRAIN EDMONSTON-B	GMYGGTYLVEK	2845	AAC35876.2
1963	MEASLES VIRUS STRAIN EDMONSTON-B	GPPISLERLDVGTN	2846	P69354.1
1964	MEASLES VIRUS STRAIN EDMONSTON-B	GPRQAQVSFL	2847	Q89933.1
1965	MEASLES VIRUS STRAIN EDMONSTON-B	GRLVPQVRVID	2848	AAF85695.1
1966	MEASLES VIRUS STRAIN EDMONSTON-B	GSAPISMGFR	2849	AAF85692.1
1967	MEASLES VIRUS STRAIN EDMONSTON-B	HILAKSTAL	2850	AAF85698.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1968	MEASLES VIRUS STRAIN EDMONSTON-B	HYREVNLVY	2851	AAF85698.1
1969	MEASLES VIRUS STRAIN EDMONSTON-B	IPPMKNLAL	2852	AAC35876.2
1970	MEASLES VIRUS STRAIN EDMONSTON-B	IPYQGSGKGVSF	2853	CAB43772.1
1971	MEASLES VIRUS STRAIN EDMONSTON-B	ISKESQHVY	2854	AAF85698.1
1972	MEASLES VIRUS STRAIN EDMONSTON-B	IVSSHFFVY	2855	AAF85698.1
1973	MEASLES VIRUS STRAIN EDMONSTON-B	KEIKETGRLF	2856	AAF85698.1
1974	MEASLES VIRUS STRAIN EDMONSTON-B	KESQHVYYL	2857	AAF85698.1
1975	MEASLES VIRUS STRAIN EDMONSTON-B	KIIDNTEQL	2858	AAF85695.1
1976	MEASLES VIRUS STRAIN EDMONSTON-B	KKQINRQN	2859	AAA63285.1
1977	MEASLES VIRUS STRAIN EDMONSTON-B	KKVDTNFIYQ	2860	AAF85698.1
1978	MEASLES VIRUS STRAIN EDMONSTON-B	KLIDGFFPA	2861	AAF85698.1
1979	MEASLES VIRUS STRAIN EDMONSTON-B	KPNLSSKRSEL	2862	BAB39848.1
1980	MEASLES VIRUS STRAIN EDMONSTON-B	KVSPYLFTV	2863	AAR89413.1
1981	MEASLES VIRUS STRAIN EDMONSTON-B	LETRTTNQFL	2864	CAB43772.1
1982	MEASLES VIRUS STRAIN EDMONSTON-B	LLKEATEL	2865	AAF85695.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1983	MEASLES VIRUS STRAIN EDMONSTON-B	LLKKGNSLY	2866	AAF85698.1
1984	MEASLES VIRUS STRAIN EDMONSTON-B	LPAPIGGMNY	2867	AAF85698.1
1985	MEASLES VIRUS STRAIN EDMONSTON-B	MPEETLHQVM	2868	AAF85698.1
1986	MEASLES VIRUS STRAIN EDMONSTON-B	PTTIRGQFS	2869	CAB43772.1
1987	MEASLES VIRUS STRAIN EDMONSTON-B	QEISRHQALGY	2870	P03424.1
1988	MEASLES VIRUS STRAIN EDMONSTON-B	RITHVDTESY	2871	P69354.1
1989	MEASLES VIRUS STRAIN EDMONSTON-B	RPGLKPDL	2872	P69354.1
1990	MEASLES VIRUS STRAIN EDMONSTON-B	RPIYGLEV	2873	AAF85698.1
1991	MEASLES VIRUS STRAIN EDMONSTON-B	RQAGQEMILAV	2874	P69354.1
1992	MEASLES VIRUS STRAIN EDMONSTON-B	SAVRIATVY	2875	AAF85698.1
1993	MEASLES VIRUS STRAIN EDMONSTON-B	SLMPEETLHQV	2876	AAF85698.1
1994	MEASLES VIRUS STRAIN EDMONSTON-B	SMIDLVTKF	2877	AAF85698.1
1995	MEASLES VIRUS STRAIN EDMONSTON-B	SMLNSQAIDNLRA	2878	P69354.1
1996	MEASLES VIRUS STRAIN EDMONSTON-B	SMYRVFEV	2879	CAB43772.1
1997	MEASLES VIRUS STRAIN EDMONSTON-B	SQQGMFHAY	2880	AAF85698.1

NO.	DISEASE	EPITOPE AMINO ACID SEQUENCE	SEQ ID NO	ACCESS. NO.
1998	MEASLES VIRUS STRAIN EDMONSTON-B	TDTPIVYNDRNLLD	2881	Q89933.1
1999	MEASLES VIRUS STRAIN EDMONSTON-B	VIINDDQGLFKV	2882	AAF85695.1
2000	MEASLES VIRUS STRAIN EDMONSTON-B	YESGVRIASL	2883	AAF85698.1
2001	MEASLES VIRUS STRAIN EDMONSTON-B	YLKDKALA	2884	AAF85698.1
2002	MEASLES VIRUS STRAIN EDMONSTON-B	YVYDHSGEAVK	2885	AAF85692.1
2003	RUBELLA VIRUS	ARVIDPAAQSFTGVV	2886	BAA28178.1
2004	RUBELLA VIRUS	SDRASARVIDPAAQS	2887	BAA28178.1
2005	RUBELLA VIRUS	VPPGKFVTAALLNTP	2888	BAA28178.1
2006	RUBELLA VIRUS	WVTPVIGSQARKCGL	2889	BAA28178.1
2007	MUMPS RUBULAVIRUS	GTYRLIPNARANLTA	400	AGC97176.1

I. Delivery of prime editors

[0820] In another aspect, the present disclosure provides for the delivery of prime editors *in vitro* and *in vivo* using various strategies, including on separate vectors using split inteins and as well as direct delivery strategies of the ribonucleoprotein complex (i.e., the prime editor complexed to the PEgRNA and/or the second-site gRNA) using techniques such as electroporation, use of cationic lipid-mediated formulations, and induced endocytosis methods using receptor ligands fused to to the ribonucleotprotein complexes. Any such methods are contemplated herein.

Overview of delivery options

[0821] In some aspects, the invention provides methods comprising delivering one or more prime editor-encoding polynucleotides, such as or one or more vectors as described herein encoding one or more components of the prime editing system described herein, one or more transcripts thereof, and/or one or proteins transcribed therefrom, to a host cell. In some aspects, the invention further provides cells produced by such methods, and organisms (such

as animals, plants, or fungi) comprising or produced from such cells. In some embodiments, a prime editor as described herein in combination with (and optionally complexed with) a guide sequence is delivered to a cell. Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding components of a prime editor to cells in culture, or in a host organism. Non-viral vector delivery systems include DNA plasmids, RNA (e.g. a transcript of a vector described herein), naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson, Science 256:808-813 (1992); Nabel & Felgner, TIBTECH 11:211-217 (1993); Mitani & Caskey, TIBTECH 11:162-166 (1993); Dillon, TIBTECH 11:167-175 (1993); Miller, Nature 357:455-460 (1992); Van Brunt, Biotechnology 6(10):1149-1154 (1988); Vigne, Restorative Neurology and Neuroscience 8:35-36 (1995); Kremer & Perricaudet, British Medical Bulletin 51(1):31-44 (1995); Haddada et al., in Current Topics in Microbiology and Immunology Doerfler and Bihm (eds) (1995); and Yu et al., Gene Therapy 1:13-26 (1994).

[0822] Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g. *in vitro* or *ex vivo* administration) or target tissues (e.g. *in vivo* administration).

[0823] The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[0824] The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and

trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (*in vivo*) or they can be used to treat cells *in vitro*, and the modified cells may optionally be administered to patients (*ex vivo*). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

[0825] The tropism of a viruses can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739 (1992); Johann et al., J. Virol. 66:1635-1640 (1992); Sommnerfelt et al., Virol. 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-2378 (1989); Miller et al., J. Virol. 65:2220-2224 (1991); PCT/US94/05700). In applications where transient expression is preferred, adenoviral based systems may be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors may also be used to transduce cells with target nucleic acids, e.g., in the *in vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy procedures (see, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et al., Mol. Cell. Biol. 4:2072-2081 (1984);

Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989).

[0826] Packaging cells are typically used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and ψ2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions are typically supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line may also be infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV. Additional methods for the delivery of nucleic acids to cells are known to those skilled in the art. See, for example, US20030087817, incorporated herein by reference.

[0827] In various embodiments, the PE constructs (including, the split-constructs) may be engineered for delivery in one or more rAAV vectors. An rAAV as related to any of the methods and compositions provided herein may be of any serotype including any derivative or pseudotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 2/1, 2/5, 2/8, 2/9, 3/1, 3/5, 3/8, or 3/9). An rAAV may comprise a genetic load (i.e., a recombinant nucleic acid vector that expresses a gene of interest, such as a whole or split PE fusion protein that is carried by the rAAV into a cell) that is to be delivered to a cell. An rAAV may be chimeric.

[0828] As used herein, the serotype of an rAAV refers to the serotype of the capsid proteins of the recombinant virus. Non-limiting examples of derivatives and pseudotypes include rAAV2/1, rAAV2/5, rAAV2/8, rAAV2/9, AAV2-AAV3 hybrid, AAVrh.10, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV-HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8, AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShH10, AAV2 (Y->F), AAV8 (Y733F),

AAV2.15, AAV2.4, AAVM41, and AAVr3.45. A non-limiting example of derivatives and pseudotypes that have chimeric VP1 proteins is rAAV2/5-1VP1u, which has the genome of AAV2, capsid backbone of AAV5 and VP1u of AAV1. Other non-limiting example of derivatives and pseudotypes that have chimeric VP1 proteins are rAAV2/5-8VP1u, rAAV2/9-1VP1u, and rAAV2/9-8VP1u.

[0829] AAV derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699-708. doi: 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan A1, Schaffer DV, Samulski RJ.). Methods for producing and using pseudotyped rAAV vectors are known in the art (see, e.g., Duan et al., J. Virol., 75:7662-7671, 2001; Halbert et al., J. Virol., 74:1524-1532, 2000; Zolotukhin et al., Methods, 28:158-167, 2002; and Auricchio et al., Hum. Molec. Genet., 10:3075-3081, 2001).

[0830] Methods of making or packaging rAAV particles are known in the art and reagents are commercially available (see, e.g., Zolotukhin et al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods 28 (2002) 158–167; and U.S. Patent Publication Numbers US20070015238 and US20120322861, which are incorporated herein by reference; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, a plasmid comprising a gene of interest may be combined with one or more helper plasmids, e.g., that contain a rep gene (e.g., encoding Rep78, Rep68, Rep52 and Rep40) and a cap gene (encoding VP1, VP2, and VP3, including a modified VP2 region as described herein), and transfected into a recombinant cells such that the rAAV particle can be packaged and subsequently purified.

[0831] Recombinant AAV may comprise a nucleic acid vector, which may comprise at a minimum: (a) one or more heterologous nucleic acid regions comprising a sequence encoding a protein or polypeptide of interest or an RNA of interest (e.g., a siRNA or microRNA), and (b) one or more regions comprising inverted terminal repeat (ITR) sequences (e.g., wild-type ITR sequences or engineered ITR sequences) flanking the one or more nucleic acid regions (e.g., heterologous nucleic acid regions). Herein, heterologous nucleic acid regions comprising a sequence encoding a protein of interest or RNA of interest are referred to as genes of interest.

[0832] Any one of the rAAV particles provided herein may have capsid proteins that have amino acids of different serotypes outside of the VP1u region. In some embodiments, the serotype of the backbone of the VP1 protein is different from the serotype of the ITRs and/or

the Rep gene. In some embodiments, the serotype of the backbone of the VP1 capsid protein of a particle is the same as the serotype of the ITRs. In some embodiments, the serotype of the backbone of the VP1 capsid protein of a particle is the same as the serotype of the Rep gene. In some embodiments, capsid proteins of rAAV particles comprise amino acid mutations that result in improved transduction efficiency.

[0833] In some embodiments, the nucleic acid vector comprises one or more regions comprising a sequence that facilitates expression of the nucleic acid (e.g., the heterologous nucleic acid), e.g., expression control sequences operatively linked to the nucleic acid. Numerous such sequences are known in the art. Non-limiting examples of expression control sequences include promoters, insulators, silencers, response elements, introns, enhancers, initiation sites, termination signals, and poly(A) tails. Any combination of such control sequences is contemplated herein (e.g., a promoter and an enhancer).

[0834] Final AAV constructs may incorporate a sequence encoding the PEgRNA. In other embodiments, the AAV constructs may incorporate a sequence encoding the second-site nicking guide RNA. In still other embodiments, the AAV constructs may incorporate a sequence encoding the second-site nicking guide RNA and a sequence encoding the PEgRNA.

[0835] In various embodiments, the PEgRNAs and the second-site nicking guide RNAs can be expressed from an appropriate promoter, such as a human U6 (hU6) promoter, a mouse U6 (mU6) promoter, or other appropriate promoter. The PEgRNAs and the second-site nicking guide RNAs can be driven by the same promoters or different promoters.

[0836] In some embodiments, a rAAV constructs or the herein compositions are administered to a subject enterally. In some embodiments, a rAAV constructs or the herein compositions are administered to the subject parenterally. In some embodiments, a rAAV particle or the herein compositions are administered to a subject subcutaneously, intraocularly, intravitreally, subretinally, intravenously (IV), intracerebro-ventricularly, intramuscularly, intrathecally (IT), intracisternally, intraperitoneally, via inhalation, topically, or by direct injection to one or more cells, tissues, or organs. In some embodiments, a rAAV particle or the herein compositions are administered to the subject by injection into the hepatic artery or portal vein.

Split PE vector-based strategies

[0837] In this aspect, the prime editors can be divided at a split site and provided as two halves of a whole/complete prime editor. The two halves can be delivered to cells (e.g., as

expressed proteins or on separate expression vectors) and once in contact inside the cell, the two halves form the complete prime editor through the self-splicing action of the inteins on each prime editor half. Split intein sequences can be engineered into each of the halves of the encoded prime editor to facilitate their transplicing inside the cell and the concomitant restoration of the complete, functioning PE.

[0838] These split intein-based methods overcome several barriers to *in vivo* delivery. For example, the DNA encoding prime editors is larger than the rAAV packaging limit, and so requires special solutions. One such solution is formulating the editor fused to split intein pairs that are packaged into two separate rAAV particles that, when co-delivered to a cell, reconstitute the functional editor protein. Several other special considerations to account for the unique features of prime editing are described, including the optimization of second-site nicking targets and properly packaging prime editors into virus vectors, including lentiviruses and rAAV.

[0839] In this aspect, the prime editors can be divided at a split site and provided as two halves of a whole/complete prime editor. The two halves can be delivered to cells (e.g., as expressed proteins or on separate expression vectors) and once in contact inside the cell, the two halves form the complete prime editor through the self-splicing action of the inteins on each prime editor half. Split intein sequences can be engineered into each of the halves of the encoded prime editor to facilitate their transplicing inside the cell and the concomitant restoration of the complete, functioning PE.

[0840] FIG. 66 depicts depicts one embodiment of a prime editor being provided as two PE half proteins which regenerate as whole prime editor through the self-splicing action of the split-intein halves located at the end or beginning of each of the prime editor half proteins. As used herein, the term "PE N-terminal half" refers to the N-terminal half of a complete prime editor and which comprises the "N intein" at the C-terminal end of the PE N-terminal half (i.e., the N-terminal extein) of the complete prime editor. The "N intein" refers to the N-terminal half of a complete, fully-formed split-intein moiety. As used herein, the term "PE C-terminal half" refers to the C-terminal half of a complete prime editor and which comprises the "C intein" at the N-terminal end of the C-terminal half (i.e., the C-terminal extein) of a complete prime editor. When the two half proteins, i.e., the PE N-terminal half and the PE C-terminal half, come into contact with one another, e.g., within the cell, the N intein and the C intein undergo the simultanous process of self-excision and the formation of a peptide bond between the C-terminal end of the PE N-terminal half and the N-terminal end of the PE C-

terminal half to reform the complete prime editor protein comprising the complete napDNAbp domain (e.g., Cas9 nickase) and the RT domain. Although not shown in the drawing, the prime editor may also comprise additional sequences including NLS at the N-terminus and/or C-terminus, as well as amino acid linkers sequences joining each domain.

[0841] In various embodiments, the prime editors may be engineered as two half proteins (i.e., a PE N-terminal half and a PE C-terminal half) by "splitting" the whole prime editor as a "split site." The "split site" refers to the location of insertion of split intein sequences (i.e., the N intein and the C intein) between two adjacent amino acid residues in the prime editor. More specifically, the "split site" refers to the location of dividing the whole prime editor into two separate halves, wherein in each halve is fused at the split site to either the N intein or the C intein motifs. The split site can be at any suitable location in the prime editor fusion protein, but preferably the split site is located at a position that allows for the formation of two half proteins which are appropriately sized for delivery (e.g., by expression vector) and wherein the inteins, which are fused to each half protein at the split site termini, are available to sufficiently interact with one another when one half protein contacts the other half protein inside the cell.

[0842] In some embodiments, the split site is located in the napDNAbp domain. In other embodiments, the split site is located in the RT domain. In other embodiments, the split site is located in a linker that joins the napDNAbp domain and the RT domain.

[0843] In various embodiments, split site design requires finding sites to split and insert an N- and C- terminal intein that are both structurally permissive for purposes of packaging the two half prime editor domains into two different AAV genomes. Additionally, intein residues necessary for trans splicing can be incorporated by mutating residues at the N terminus of the C terminal extein or inserting residues that will leave an intein "scar."

[0844] Exemplary split configurations of split prime editors comprising either the SpCas9 nickase or the SaCas9 nickase are as follows.

S. PYOGENES PE, SPLIT AT LINKER, N TERMINAL PORTION STRUCTURE: [N EXTEIN]-[N INTEIN]
MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLG** **NTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEM** **AKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVD** **STDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE** **ENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPN** **FKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDI** **LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG** **YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIP**

HQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWM
TRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYF
TVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYF
KKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLT
LFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSG
KTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAG
SPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERM
KRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLS
DYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQL
LNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRM
NTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAV
VGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNF
FKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKT
EVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFE
LENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQL
FVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGG
DSGGSSGGSCLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWH
DRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPNSG
GSKRTADGSEFEPKKKRKV(SEQ ID NO: 443)

KEY:
NLS (SEQ ID NO: 124, 125)
CAS9 (SEQ ID NO: 445)
LINKER (SEQ ID NO: 446)
NPUN INTEIN (SEQ ID NO: 447)

S. PYOGENES PE, SPLIT AT LINKER, C TERMINAL PORTION
STRUCTURE: [C INTEIN]-[C EXTEIN]

MKRTADGSEFESPKKKRKV*IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN***CFNS**
GSETPGTSESATPESSGGSSGGSS*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWA*
ETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPW
NTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKD
AFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRI
QHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGY
LLKEGQRWLTEARKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKT
GTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW
RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQP
PDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTR
PDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIA
LTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFL
*PKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP***SGGS**KRTA
DGSEFEPKKKRKV(SEQ ID NO: 450)

KEY:
NLS (SEQ ID NO: 124, 125)

LINKER 1 (SEQ ID NO: 453)
LINKER 2 (SEQ ID NO: 174)

NPUC INTEIN (SEQ ID NO: 452)
RT (SEQ ID NO: 454)

S. AUREUS PE, SPLIT BETWEEN RESIDUES 740/741, N TERMINAL PORTION STRUCTURE: [N EXTEIN]-[N INTEIN]

MKRTADGSEFESPKKKRKV**GKRNYILGLAIGITSVGYGIIDYETRDVIDAGVRLF KEANVENNEGRRSKRGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINP YEARVKGLSQKLSEEEFSAALLHLAKRRGVHNVNEVEEDTGNELSTKEQISRN SKALEEKYVAELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQL DQSFIDTYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELR SVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQ IAKEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQIAKI LTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAINLILDELWH TNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAII KKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGKENAK YLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHIIPRSVSFDNSFNNKV LVKQEENSKKGNRTPFQYLSSSDSKISYETFKKHILNLAKGKGRISKTKKEYLL EERDINRFSVQKDFINRNLVDTRYATRGLMNLLRSYFRVNNLDVKVKSINGGF TSFLRRKWKFKKERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQ MFEEKQAE**CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQWH DRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN**SG GS**KRTADGSEFEPKKKRKV(SEQ ID NO: 458)

KEY:
NLS (SEQ ID NO: 124, 125)
CAS9 (SEQ ID NO: 460)
LINKER (SEQ ID NO: 174)
NPUN INTEIN (SEQ ID NO: 462)

S. AUREUS PE, SPLIT BETWEEN RESIDUES 740/741, C TERMINAL PORTION STRUCTURE: [C INTEIN]-[C EXTEIN]

MKRTADGSEFESPKKKRKV*IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN***CFNE**IETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKKPNRKLINDTLYSTRKDDKG NTLIVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGD EKNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRN KVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEAKKLK KISNQAEFIASFYKNDLIKINGELYRVIGVNNDLLNRIEVNMIDITYREYLENMN DKRPPHIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG**SGGSSGGSSGS ETPGTSESATPESSGGSSGGSS***TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAET GGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNT PLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAF FCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQ HPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYL LKEGQRWLTEARKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKT

*GTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPW RRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQP PDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTR PDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIA LTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFL PKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP***SGGS***KRTA* DGSEFEPKKKRKV(SEQ ID NO: 465)

KEY:
NLS (SEQ ID NO: 124, 125)
CAS9 (SEQ ID NO: 467)
LINKER 1 (SEQ ID NO: 127)
LINKER 2 (SEQ ID NO: 174)
NPUC INTEIN (SEQ ID NO: 452)
RT (SEQ ID NO: 471)

[0845] In various embodiments, using SpCas9 nickase (SEQ ID NO: 18, 1368 amino acids) as an example, the split can between between any two amino acids between 1 and 1368. Preferred splits, however, will be located between the central region of the protein, e.g., from amino acids 50-1250, or from 100-1200, or from 150-1150, or from 200-1100, or from 250-1050, or from 300-1000, or from 350-950, or from 400-900, or from 450-850, or from 500-800, or from 550-750, or from 600-700 of SEQ ID NO: 18. In specific exemplary embodiments, the split site may be between 740/741, or 801/802, or 1010/1011, or 1041/1042. In other embodiments the split site may be between 1/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 10/11, 12/13, 14/15, 15/16, 17/18, 19/20, 20/21, 21/22, 22/23, 23/24, 24/25, 25/26, 26/27, 27/28, 28/29, 29/30, 30/31, 31/32, 32/33, 33/34, 34/35, 35/36, 36/37, 38/39, 39/40, 41/42, 42/43, 43/44, 44/45, 45/46, 46/47, 47/48, 48/49, 49/50, 51/52, 52/53, 53/54, 54/55, 55/56, 56/57, 57/58, 58/59, 59/60, 61/62, 62/63, 63/64, 64/65, 65/66, 66/67, 67/68, 68/69, 69/70, 71/72, 72/73, 73/74, 74/75, 75/76, 76/77, 77/78, 78/79, 79/80, 81/82, 82/83, 83/84, 84/85, 85/86, 86/87, 87/88, 88/89, 89/90, or between any two pairs of adjacent residues between 90-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, 1150-1200, 1200-1250, 1250-1300, 1300-1350, and 1350-1368, relative to SpCas9 of SEQ ID NO: 18, at between any two corresponding residues in an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.9% sequence identity with SEQ ID NO: 18, or between any two corresponding residues in a variant or equivalent of SpCas9 of any of amino acid sequences SEQ ID NOs. 19-88, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.9% sequence identity with any of SEQ ID NOs: 19-88.

[0846] In various embodiments, the split intein sequences can be engineered by from the following intein sequences.

NAME	SEQUENCE OF LIGAND-DEPENDENT INTEIN
2-4 INTEIN:	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 472)
3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGDR VAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEASM MGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWLEIL MIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLATS SRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRAL DKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEHL YSMKYTNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDDK FLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVHN C (SEQ ID NO: 473)
30R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 474)
30R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 475)
30R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPIPYSEYDPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLECAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL

	ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC (SEQ ID NO: 476)
37R3-1 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKG DRVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYNPTSPFSEA SMMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWL EILMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLL ATSSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIH RALDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGM EHLYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADAL DDKFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVV VHNC ((SEQ ID NO: 477)
37R3-2 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAAAKDGTLLARPVV SWFDQGTRDVIGLRIAGGAIVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEGLRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 478)
37R3-3 INTEIN	CLAEGTRIFDPVTGTTHRIEDVVDGRKPIHVVAVAKDGTLLARPVVS WFDQGTRDVIGLRIAGGATVWATPDHKVLTEYGWRAAGELRKGD RVAGPGGSGNSLALSLTADQMVSALLDAEPPILYSEYDPTSPFSEAS MMGLLTNLADRELVHMINWAKRVPGFVDLTLHDQAHLLERAWLEI LMIGLVWRSMEHPGKLLFAPNLLLDRNQGKCVEGMVEIFDMLLAT SSRFRMMNLQGEEFVCLKSIILLNSGVYTFLSSTLKSLEEKDHIHRA LDKITDTLIHLMAKAGLTLQQQHQRLAQLLLILSHIRHMSNKGMEH LYSMKYKNVVPLYDLLLEMLDAHRLHAGGSGASRVQAFADALDD KFLHDMLAEELRYSVIREVLPTRRARTFDLEVEELHTLVAEGVVVH NC (SEQ ID NO: 479)

[0847] In various other embodiments, the split intein sequences can be used as follows:

INTEIN-N	INTEIN-C
NPU-N CLSYETEILTVEYGLLPIGKIVEKRIEC TVYSVDNNGNIYTQPVAQWHDRGEQ EVFEYCLEDGSLIRATKDHKFMTVDG QMLPIDEIFERELDLMRVDNLPNSGG S (SEQ ID NO: 447)	NPU-C IKIATRKYLGKQNVYDIGVERDHNFALKNG FIASN (SEQ ID NO: 452)

[0848] In various embodiments, the split inteins can be used to separately deliver separate portions of a complete PE fusion protein to a cell, which upon expression in a cell, become reconstituted as a complete PE fusion protein through the trans splicing.

[0849] In some embodiments, the disclosure provides a method of delivering a PE fusion protein to a cell, comprising:

(a) constructing a first expression vector encoding an N-terminal fragment of the PE fusion protein fused to a first split intein sequence;

(b) constructing a second expression vector encoding a C-terminal fragment of the PE fusion protein fused to a second split intein sequence;

(c) delivering the first and second expression vectors to a cell,

wherein the N-terminal and C-terminal fragment are reconstituted as the PE fusion protein in the cell as a result of trans splicing activity causing self-excision of the first and second split intein sequences.

[0850] The split site in some embodiments can be anywhere in the prime editor fusion, including the napDNAbp domain, the linker, or the reverse transcriptase domain.

[0851] In other embodiments, the split site is in the napDNAbp domain.

[0852] In still other embodiments, the split site is in the reverse transcriptase or polymerase domain.

[0853] In yet other embodiments, the split site is in the linker.

[0854] In various embodiments, the present disclosure provides prime editors comprising a napDNAbp (e.g., a Cas9 domain) and a reverse transcriptase wherein one or both of the napDNAbp and/or the reverse transcriptase comprise an intein, for example, a ligand-dependent intein. Typically the intein is a ligand-dependent intein which exhibits no or minimal protein splicing activity in the absence of ligand (e.g., small molecules such as 4-hydroxytamoxifen, peptides, proteins, polynucleotides, amino acids, and nucleotides). Ligand-dependent inteins are known, and include those described in U.S. patent application, U.S.S.N. 14/004,280, published as U.S. 2014/0065711 A1, the entire contents of which are incorporated herein by reference. In addition, use of split-Cas9 architecture In some embodiments, the intein comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-15, 447, 452, 462, and 472-479.

[0855] In various embodiments, the napDNAbp domains are smaller-sized napDNAbp domains as compared to the canonical SpCas9 domain of SEQ ID NO: 18.

[0856] The canonical SpCas9 protein is 1368 amino acids in length and has a predicted molecular weight of 158 kilodaltons. The term "small-sized Cas9 variant", as used herein, refers to any Cas9 variant—naturally occurring, engineered, or otherwise—that is less than at least 1300 amino acids, or at least less than 1290 amino acids, or than less than 1280 amino

acids, or less than 1270 amino acid, or less than 1260 amino acid, or less than 1250 amino acids, or less than 1240 amino acids, or less than 1230 amino acids, or less than 1220 amino acids, or less than 1210 amino acids, or less than 1200 amino acids, or less than 1190 amino acids, or less than 1180 amino acids, or less than 1170 amino acids, or less than 1160 amino acids, or less than 1150 amino acids, or less than 1140 amino acids, or less than 1130 amino acids, or less than 1120 amino acids, or less than 1110 amino acids, or less than 1100 amino acids, or less than 1050 amino acids, or less than 1000 amino acids, or less than 950 amino acids, or less than 900 amino acids, or less than 850 amino acids, or less than 800 amino acids, or less than 750 amino acids, or less than 700 amino acids, or less than 650 amino acids, or less than 600 amino acids, or less than 550 amino acids, or less than 500 amino acids, but at least larger than about 400 amino acids and retaining the required functions of the Cas9 protein.

[0857] In one embodiment, as depicted in Example 20, the specification embraces the following split-intein PE contructs, which are split between residues 1024 and 1025 of the canonical SpCas9 (SEQ ID NO: 18) (or which may be referred to as residues 1023 and 1024, respectively, relative to a Met-minus SEQ ID NO: 18).

[0858] First, the amino acid sequence of SEQ ID NO: 18 is shown as follows, indicating the location of the split site between 1024 ("K") and 1025 ("S") residues:

Description	**Sequence**	SEQ ID NO:
SpCas9 *Streptococcus pyogenes* M1 SwissProt Accession No. Q99ZW2 Wild type	MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADL RLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQ TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDI LRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH AILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGN SRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTN FDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGM RKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEE NEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV	SEQ ID NO: 18, indicated with split site 1024/1025 in bold The M at position 1 is not necessarily present in the PE fusion protein in certain embodiments. Thus, the numbering of the split site is 1023/1024 in the case that the amino acid sequence

	ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEV VKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHD AYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA **KS**EQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLI ETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPT VAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEK NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRML ASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPED NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDT TIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD	excludes Met at position 1.

[0859] In this configuration, the amino acid sequence of N-terminal half (amino acids 1-1024) is as follows:

[0860] MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEED KKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGH FLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLI AQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIG DQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQ QLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARG NSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLY EYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKK IECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREM IEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGF ANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVD ELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVE NTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLT RSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDK AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQ FYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA**K** (SEQ ID NO: 3877).

[0861] In this configuration, the amino acid sequence of N-terminal half (amino acids 1-1023) (where the protein is Met-minus at position 1) is as follows:

[0862] DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFD SGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDK KHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFL IEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQ LPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQ YADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQL PEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRK QRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNS RFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEY FTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIE CFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIE ERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFA NRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDE LVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVEN TQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTR SDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKA GFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQF YKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIA**K** (SEQ ID NO: 3878).

[0863] In this configuration, the amino acid sequence of C-terminal half (amino acids 1024-1368 (or counted as amino acids 1023-1367 in a Met-minus Cas9) is as follows:

[0864] **S**EQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGR DFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGF DSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVK KDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQA ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLG GD (SEQ ID NO: 3879).

[0865] As shown in Example 20, the PE2 (which is based on SpCas9 of SEQ ID NO: 18) construct was split at position 1023/1024 (relative to a Met-minus SEQ ID NO: 18) into two separate constructs, as follows:

[0866] SpPE2 split at 1023/1024 N terminal half

[0867] MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKV
LGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAK
VDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDA
KAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS
KDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK
MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKI
EKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNF
DKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK
TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN
EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR
IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDA
IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRK
FDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREV
KVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY
GDYKVYDVRKMIAK*CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQW*
*HDRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN***SGGS**
KRTADGSEFEPKKKRKV (SEQ ID NO: 3875)

[0868] Key: NLS, Cas9, Mutated residues, **Linker**, *NpuN intein*, *NpuC intein*, **RT**

[0869] SpPE2 split at 1023/1024 C terminal half

MKRTADGSEFESPKKKRKV*IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN*CFNEIG
KATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLS
MPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV
VAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF
ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVE
QHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLG
APAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD**SGGSSGGSS**
GSETPGTSESATPESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA
WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGIL
VPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPP
SHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKN
SPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLG
NLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQL
REFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLT
APALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAG

WPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARM THYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELI ALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEI LALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLI ENSSPSGGS*KRTADGSEFEPKKKRK*** (SEQ ID NO: 3876)**

[0870] Key: NLS, Cas9, Mutated residues, **Linker**, *NpuN intein*, *NpuC intein*, **RT**

[0871] The present disclosure also contemplates methods of delivering split-intein prime editors to cells and/or treating cells with split-intein prime editors.

[0872] In some embodiments, the disclosure provides a method of delivering a PE fusion protein to a cell, comprising:

(a) constructing a first expression vector encoding an N-terminal fragment of the PE fusion protein fused to a first split intein sequence;

(b) constructing a second expression vector encoding a C-terminal fragment of the PE fusion protein fused to a second split intein sequence;

(c) delivering the first and second expression vectors to a cell,

wherein the N-terminal and C-terminal fragment are reconstituted as the PE fusion protein in the cell as a result of trans splicing activity causing self-excision of the first and second split intein sequences.

[0873] In certain embodiments, the N-terminal fragment of the PE fusion protein fused to a first split intein sequence is SEQ ID NO: 3875, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99.9% sequence identity with SEQ ID NO: 3875.

[0874] In other embodiments, the C-terminal fragment of the PE fusion protein fused to a first split intein sequence is SEQ ID NO: 3876, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99.9% sequence identity with SEQ ID NO: 3876.

[0875] In other embodiments, the disclosure provides a method of editing a target DNA sequence within a cell, comprising:

(a) constructing a first expression vector encoding an N-terminal fragment of the PE fusion protein fused to a first split intein sequence;

(b) constructing a second expression vector encoding a C-terminal fragment of the PE fusion protein fused to a second split intein sequence;

(c) delivering the first and second expression vectors to a cell,

wherein the N-terminal and C-terminal fragment are reconstituted as the PE fusion protein in the cell as a result of trans splicing activity causing self-excision of the first and second split intein sequences.

[0876] In certain embodiments, the N-terminal fragment of the PE fusion protein fused to a first split intein sequence is SEQ ID NO: 3875, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99.9% sequence identity with SEQ ID NO: 3875.

[0877] In other embodiments, the C-terminal fragment of the PE fusion protein fused to a first split intein sequence is SEQ ID NO: 3876, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99.9% sequence identity with SEQ ID NO: 3876.

Delivery of PE ribonucleoprotein complexes

[0878] In this aspect, the prime editors may be delivered by non-viral delivery strategies involving delivery of a prime editor complexed with a PEgRNA (i.e., a PE ribonucleoprotein complex) by various methods, including electroporation and lipid nanoparticles. Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g. *in vitro* or *ex vivo* administration) or target tissues (e.g. *in vivo* administration).

[0879] The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[0880] Additional reference may be made to the following references that discuss approaches for non-viral delivery of ribonucleoprotein complexes, each of which are incorporated herein by reference.

[0881] Chen, Sean, et al. "Highly efficient mouse genome editing by CRISPR ribonucleoprotein electroporation of zygotes." *Journal of Biological Chemistry* (2016): jbc-M116. PubMed

[0882] Zuris, John A., et al. "Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo." *Nature biotechnology* 33.1 (2015): 73. PubMed

[0883] Rouet, Romain, et al. "Receptor-Mediated Delivery of CRISPR-Cas9 Endonuclease for Cell-Type-Specific Gene Editing." *Journal of the American Chemical Society* 140.21 (2018): 6596-6603. PubMed.

[0884] FIG. 68C provides data showing that various disclosed PE ribonucleoprotein complexes (PE2 at high concentration, PE3 at high concentration and PE3 at low concentration) can be delivered in this manner.

Delivery of PE by mRNA

[0885] Another method that may be employed to deliver prime editors and/or PEgRNAs to cells in which prime editing-based genome editing is desired is by employing the use of messenger RNA (mRNA) delivery methods and technologies. Examples of mRNA delivery methods and compositions that may be utilized in the present disclosure including, for example, PCT/US2014/028330, US8822663B2, NZ700688A, ES2740248T3, EP2755693A4, EP2755986A4, WO2014152940A1, EP3450553B1, BR112016030852A2, and EP3362461A1, each of which are incorporated herein by reference in their entireties. Additional disclosure hereby incorporated by reference can be found in Kowalski et al., "Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery," *Mol Therap.*, 2019; 27(4): 710-728.

[0886] In contrast to DNA vector encoding prime editors, the use of RNA as delivery agent for prime editors has the advantage that the genetic material does not have to enter the nucleus to perform its function. The delivered mRNA may be directly translated in the cytoplasm into the desired protein (e.g., prime editor fusion protein) and nucleic acid products (e.g., PEgRNA). However, in order to be more stable (e.g., resist RNA-degrading enzymes in the cytoplasm), it is in some embodiments necessary to stabilize the mRNA to improve delivery efficiency. Certain delivery carriers such as cationic lipids or polymeric delivery carriers can also help protect the transfected mRNA from endogenous RNase enzymes that might otherwise degrade the therapeutic mRNA encoding the desired prime editor fusion proteins. In addition, despite the increased stability of modified mRNA,

delivery of mRNA, particularly mRNA encoding full-length protein, to cells in vivo in a manner that allows therapeutic levels of protein production remains a challenge.

[0887] With some exceptions, the intracellular delivery of mRNA is generally more challenging than that of small oligonucleotides, and it requires encapsulation into a delivery nanoparticle, in part due to the significantly larger size of mRNA molecules (300–5,000 kDa, ~1–15 kb) as compared to other types of RNAs (small interfering RNAs [siRNAs], ~14 kDa; antisense oligonucleotides [ASOs], 4–10 kDa).

[0888] mRNA must cross the cell membrane in order to reach the cytoplasm. The cell membrane is a dynamic and formidable barrier to intracellular delivery. It is made up primarily of a lipid bilayer of zwitterionic and negatively charged phospholipids, where the polar heads of the phospholipids point toward the aqueous environment and the hydrophobic tails form a hydrophobic core.

[0889] In some embodiments, the mRNA compositions of the disclosure comprise mRNA (encoding a prime editor and/or PEgRNA), a transport vehicle, and optionally an agent that facilitates contact with the target cell and subsequent transfection.

[0890] In some embodiments, the mRNA can include one or more modifications that confer stability to the mRNA (eg, compared to the wild-type or native version of the mRNA) and is involved in the associated abnormal expression of the protein. One or more modifications to the wild type that correct the defect may also be included. For example, the nucleic acids of the invention can include modifications of one or both of a 5' untranslated region or a 3' untranslated region. Such modifications may include the inclusion of sequences encoding a partial sequence of the cytomegalovirus (CMV) immediate early 1 (IE1) gene, poly A tail, Cap1 structure, or human growth hormone (hGH). In some embodiments, the mRNA is modified to reduce mRNA immunogenicity.

[0891] In one embodiment, the "prime editor" mRNA in the composition of the invention can be formulated in a liposome transfer vehicle to facilitate delivery to target cells. Contemplated transfer vehicles can include one or more cationic lipids, non-cationic lipids, and/or PEG-modified lipids. For example, the transfer vehicle can include at least one of the following cationic lipids: C12-200, DLin-KC2-DMA, DODAP, HGT4003, ICE, HGT5000, or HGT5001. In embodiments, the transfer vehicle comprises cholesterol (chol) and / or PEG modified lipids. In some embodiments, the transfer vehicle comprises DMG-PEG2K. In certain embodiments, the transfer vehicle has the following lipid formulation: C12-200,

DOPE, chol, DMG-PEG2K; DODAP, DOPE, cholesterol, DMG-PEG2K; HGT5000, DOPE, chol, DMG-PEG2K, HGT5001, DOPE, chol, one of DMG-PEG2K.

[0892] The present disclosure also provides compositions and methods useful for facilitating transfection of target cells with one or more PE-encoding mRNA molecules. For example, the compositions and methods of the present invention contemplate the use of targeting ligands that can increase the affinity of the composition for one or more target cells. In one embodiment, the targeting ligand is apolipoprotein B or apolipoprotein E, and the corresponding target cells express low density lipoprotein receptors and thus promote recognition of the targeting ligand. A vast number of target cells can be preferentially targeted using the methods and compositions of the present disclosure. For example, contemplated target cells include hepatocytes, epithelial cells, hematopoietic cells, epithelial cells, endothelial cells, lung cells, bone cells, stem cells, mesenchymal cells, nerve cells, heart cells, adipocytes, vascular smooth muscle Includes cells, cardiomyocytes, skeletal muscle cells, beta cells, pituitary cells, synovial lining cells, ovarian cells, testis cells, fibroblasts, B cells, T cells, reticulocytes, leukocytes, granulocytes, and tumor cells However, it is not limited to these.

[0893] In some embodiments, the PE-encoding mRNA may optionally have chemical or biological modifications which, for example, improve the stability and/or half-life of such mRNA or which improve or otherwise facilitate protein production. Upon transfection, a natural mRNA in the compositions of the invention may decay with a half-life of between 30 minutes and several days. The mRNAs in the compositions of the disclosure may retain at least some ability to be translated, thereby producing a functional protein or enzyme. Accordingly, the invention provides compositions comprising and methods of administering a stabilized mRNA. In some embodiments, the activity of the mRNA is prolonged over an extended period of time. For example, the activity of the mRNA may be prolonged such that the compositions of the present disclosure are administered to a subject on a semi-weekly or bi-weekly basis, or more preferably on a monthly, bi-monthly, quarterly or an annual basis. The extended or prolonged activity of the mRNA of the present invention is directly related to the quantity of protein or enzyme produced from such mRNA. Similarly, the activity of the compositions of the present disclosure may be further extended or prolonged by modifications made to improve or enhance translation of the mRNA. Furthermore, the quantity of functional protein or enzyme produced by the target cell is a function of the quantity of mRNA delivered to the target cells and the stability of such mRNA. To the extent

that the stability of the mRNA of the present invention may be improved or enhanced, the half-life, the activity of the produced protein or enzyme and the dosing frequency of the composition may be further extended.

[0894] Accordingly, in some embodiments, the mRNA in the compositions of the disclosure comprise at least one modification which confers increased or enhanced stability to the nucleic acid, including, for example, improved resistance to nuclease digestion in vivo. As used herein, the terms "modification" and "modified" as such terms relate to the nucleic acids provided herein, include at least one alteration which preferably enhances stability and renders the mRNA more stable (e.g., resistant to nuclease digestion) than the wild-type or naturally occurring version of the mRNA. As used herein, the terms "stable" and "stability" as such terms relate to the nucleic acids of the present invention, and particularly with respect to the mRNA, refer to increased or enhanced resistance to degradation by, for example nucleases (i.e., endonucleases or exonucleases) which are normally capable of degrading such mRNA. Increased stability can include, for example, less sensitivity to hydrolysis or other destruction by endogenous enzymes (e.g., endonucleases or exonucleases) or conditions within the target cell or tissue, thereby increasing or enhancing the residence of such mRNA in the target cell, tissue, subject and/or cytoplasm. The stabilized mRNA molecules provided herein demonstrate longer half-lives relative to their naturally occurring, unmodified counterparts (e.g. the wild-type version of the mRNA). Also contemplated by the terms "modification" and "modified" as such terms related to the mRNA of the present invention are alterations which improve or enhance translation of mRNA nucleic acids, including for example, the inclusion of sequences which function in the initiation of protein translation (e.g., the Kozak consensus sequence). (Kozak, M., Nucleic Acids Res 15 (20): 8125-48 (1987)).

[0895] In some embodiments, the mRNAs used in the compositions of the disclosure have undergone a chemical or biological modification to render them more stable. Exemplary modifications to an mRNA include the depletion of a base (e.g., by deletion or by the substitution of one nucleotide for another) or modification of a base, for example, the chemical modification of a base. The phrase "chemical modifications" as used herein, includes modifications which introduce chemistries which differ from those seen in naturally occurring mRNA, for example, covalent modifications such as the introduction of modified nucleotides, (e.g., nucleotide analogs, or the inclusion of pendant groups which are not naturally found in such mRNA molecules).

[0896] Other suitable polynucleotide modifications that may be incorporated into the PE-encoding mRNA used in the compositions of the disclosure include, but are not limited to, 4'-thio-modified bases: 4'-thio-adenosine, 4'-thio-guanosine, 4'-thio-cytidine, 4'-thio-uridine, 4'-thio-5-methyl-cytidine, 4'-thio-pseudouridine, and 4'-thio-2-thiouridine, pyridin-4-one ribonucleoside, 5-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine, 3-methyluridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine, 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyluridine, 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine, 1-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio-1-methyl-pseudouridine, 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine, dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, 5-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, 2-aminopurine, 2,6-diaminopurine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine, N6-methyladenosine, N6-isopentenyladenosine, N6-(cis-hydroxyisopentenyl)adenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine, N6-glycinylcarbamoyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6,N6-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, and 2-methoxy-adenine, inosine, 1-methyl-inosine, wyosine, wybutosine, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, N2-methylguanosine, N2,N2-dimethylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, and N2,N2-dimethyl-6-thio-guanosine, and combinations thereof. The term modification also includes, for example, the incorporation of non-nucleotide linkages or modified nucleotides into the mRNA sequences

of the present invention (e.g., modifications to one or both of the 3' and 5' ends of an mRNA molecule encoding a functional protein or enzyme). Such modifications include the addition of bases to an mRNA sequence (e.g., the inclusion of a poly A tail or a longer poly A tail), the alteration of the 3' UTR or the 5' UTR, complexing the mRNA with an agent (e.g., a protein or a complementary nucleic acid molecule), and inclusion of elements which change the structure of an mRNA molecule (e.g., which form secondary structures).

[0897] In some embodiments, PE-encoding mRNAs include a 5' cap structure. A 5' cap is typically added as follows: first, an RNA terminal phosphatase removes one of the terminal phosphate groups from the 5' nucleotide, leaving two terminal phosphates; guanosine triphosphate (GTP) is then added to the terminal phosphates via a guanylyl transferase, producing a 5'5'5 triphosphate linkage; and the 7-nitrogen of guanine is then methylated by a methyltransferase. Examples of cap structures include, but are not limited to, m7G(5')ppp (5'(A,G(5')ppp(5')A and G(5')ppp(5')G. Naturally occurring cap structures comprise a 7-methyl guanosine that is linked via a triphosphate bridge to the 5'-end of the first transcribed nucleotide, resulting in a dinucleotide cap of m7G(5')ppp(5')N, where N is any nucleoside. In vivo, the cap is added enzymatically. The cap is added in the nucleus and is catalyzed by the enzyme guanylyl transferase. The addition of the cap to the 5' terminal end of RNA occurs immediately after initiation of transcription. The terminal nucleoside is typically a guanosine, and is in the reverse orientation to all the other nucleotides, i.e., G(5')ppp(5')GpNpNp.

[0898] Additional cap analogs include, but are not limited to, a chemical structures selected from the group consisting of m7GpppG, m7GpppA, m7GpppC; unmethylated cap analogs (e.g., GpppG); dimethylated cap analog (e.g., m2,7GpppG), trimethylated cap analog (e.g., m2,2,7GpppG), dimethylated symmetrical cap analogs (e.g., m7Gpppm7G), or anti reverse cap analogs (e.g., ARCA; m7,2'OmeGpppG, m72'dGpppG, m7,3'OmeGpppG, m7,3'dGpppG and their tetraphosphate derivatives) (see, e.g., Jemielity, J. et al., "Novel 'anti-reverse' cap analogs with superior translational properties", RNA, 9: 1108-1122 (2003)).

[0899] Typically, the presence of a "tail" serves to protect the mRNA from exonuclease degradation. A poly A or poly U tail is thought to stabilize natural messengers and synthetic sense RNA. Therefore, in certain embodiments a long poly A or poly U tail can be added to an mRNA molecule thus rendering the RNA more stable. Poly A or poly U tails can be added using a variety of art-recognized techniques. For example, long poly A tails can be added to synthetic or in vitro transcribed RNA using poly A polymerase (Yokoe, et al. Nature Biotechnology. 1996; 14: 1252-1256). A transcription vector can also encode long poly A

tails. In addition, poly A tails can be added by transcription directly from PCR products. Poly A may also be ligated to the 3' end of a sense RNA with RNA ligase (see, e.g., Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1991 edition)).

[0900] Typically, the length of a poly A or poly U tail can be at least about 10, 50, 100, 200, 300, 400 at least 500 nucleotides. In some embodiments, a poly-A tail on the 3' terminus of mRNA typically includes about 10 to 300 adenosine nucleotides (e.g., about 10 to 200 adenosine nucleotides, about 10 to 150 adenosine nucleotides, about 10 to 100 adenosine nucleotides, about 20 to 70 adenosine nucleotides, or about 20 to 60 adenosine nucleotides). In some embodiments, mRNAs include a 3' poly(C) tail structure. A suitable poly-C tail on the 3' terminus of mRNA typically include about 10 to 200 cytosine nucleotides (e.g., about 10 to 150 cytosine nucleotides, about 10 to 100 cytosine nucleotides, about 20 to 70 cytosine nucleotides, about 20 to 60 cytosine nucleotides, or about 10 to 40 cytosine nucleotides). The poly-C tail may be added to the poly-A or poly U tail or may substitute the poly-A or poly U tail.

[0901] PE-encoding mRNAs according to the present disclosure may be synthesized according to any of a variety of known methods. For example, mRNAs according to the present invention may be synthesized via *in vitro* transcription (IVT). Briefly, IVT is typically performed with a linear or circular DNA template containing a promoter, a pool of ribonucleotide triphosphates, a buffer system that may include DTT and magnesium ions, and an appropriate RNA polymerase (e.g., T3, T7 or SP6 RNA polymerase), DNAse I, pyrophosphatase, and/or RNAse inhibitor. The exact conditions will vary according to the specific application.

[0902] In embodiments involving mRNA delivery, the ratio of the mRNA encoding the PE fusion protein to the PEgRNA may be important for efficient editing. In certain embodiments, the weight ratio of mRNA (encoding the PE fusion protein) to PEgRNA is 1:1. In certain other embodiments, the weight ratio of mRNA (encoding the PE fusion protein) to PEgRNA is 2:1. In still other embodiments, the weight ratio of mRNA (encoding the PE fusion protein) to PEgRNA is 1:2. In still further embodiments, the weight ratio of mRNA (encoding the PE fusion protein) to PEgRNA is selected from the group consisting of about 1:1000, 1:900; 1:800; 1:700; 1:600; 1:500; 1:400; 1:300; 1:200; 1:100; 1:90; 1:80; 1:70; 1:60; 1:50; 1:40; 1:30; 1:20; 1:10; and 1:1. In other embodiments, the weight ratio of mRNA (encoding the PE fusion protein) to PEgRNA is selected from the group consisting of about

1:1000, 1:900; 800:1; 700:1; 600:1; 500:1; 400:1; 300:1; 200:1; 100:1; 90:1; 80:1; 70:1; 60:1; 50:1; 40:1; 30:1; 20:1; 10:1; and 1:1.

J. Use of prime editing for identifying off-target editing in an unbiased manner

[0903] Like other genome editors, there exists some risk that PE may introduce its programmed genetic alterations at unintended sites around the genome, i.e., "off-target" sites. However, there are currently no described methods to detect off-target editing with prime editors. Such methods would allow the identification of potential sites of off-target editing using prime editors.

[0904] The key concept of this aspect is the idea of using prime editing to insert the same adapter sequence and/or primer binding site at on-target and off-target sites, templated from the same PEgRNA, to enable the rapid identification of genomic off-target modification sites of napDNAbp nucleases or prime editors. This method is distinguished from other techniques that identify nuclease off-target sites because the adapter and/or primer binding sequence is inserted in the same event as DNA binding and nicking by the napDNAbp, simplifying the downstream processing.

[0905] FIG. 33 illustrates the basic principle of off-target identification. The figure is a schematic showing PEgRNA design for primer binding sequence insertions and primer binding insertion into genomic DNA using prime editing for determining off-target editing. In this embodiment, prime editing is conducted inside a living cell, a tissue, or an animal model. As a first step, an appropriate PEgRNA is designed. The top schematic shows a exemplary PEgRNA that may be used in this aspect. The spacer in the PEgRNA (labeled as "protospacer") is complementary to one of the strands of the genomic target. The PE:PEgRNA complex (i.e., the PE complex) installs a single stranded 3′ end flap at the nick site which contains the encoded primer binding sequence and the region of homology (coded by the homology arm of the PEgRNA) that is complementary to the region just downstream of the cut site (in red). Through flap invasion and DNA repair/replication processes, the synthesized strand becomes incorporated into the DNA, thereby installing the primer binding site. This process can occur at the desired genomic target, but also at other genomic sites that might interact with the PEgRNA in an off-target manner (i.e., the PEgRNA guides the PE complex to other off-target sites due to the complementarity of the spacer region to other genomic sites that are not the intended genomic site). Thus, the primer binding sequence may be installed not only at the deisred genomic target, but at off-target genomic sites elsewhere in the genome. In order to detect the insertion of these primer binding sites at both

the intended genomic target sites and the off-target genomic sites, the genomic DNA (post-PE) can be isolated, fragmented, and ligated to adapter nucleotides (shown in red). Next, PCR may be carried out with PCR oligonucleotides that anneal to the adapters and to the inserted primer binding sequence to amplify on-target and off-target genomic DNA regions into which the primer binding site was inserted by PE. High throughput sequencing then may be conducted, as well as sequence alignments, to identify the insertion points of PE-inserted primer binding sequences at either the on-target site or at off-target sites.

[0906] Thus, FIG. 33 illustrates one aspect regarding the identification of off-target editing sites when editing inside a living cell, in tissue culture or animal models. To conduct this method, a PEgRNA is generated that has an identical spacer to the final desired prime editor (and, if looking at prime editing off-targets, an identical primer-binding site sequence to the final desired editor), but includes the necessary sequences to install an adapter or primer binding site after reverse transcription by prime editing. *In vivo* editing is conducted using a prime editor or RT-fused nuclease, and isolate genomic DNA. The genomic DNA is fragmented by enzymatic or mechanical means and append a different adapter to sites of DNA fragmentation. PCR is used to amplify from one adapter to the adapter installed via PEgRNA. The resulting product is deep-sequenced to identify all modified sites.

[0907] In another aspect, evaluation of off-target editing by PE may be conducted *in vitro.* In this aspect, PE may be used during *in vitro* modification of genomic DNA identification of off-target editing sites using *in vitro* modification of genomic DNA. To conduct this method, ribonucleoprotein (RNP) of purified prime editor fusion protein and a PEgRNA (i.e., the PE complex) is assembled that is configured to install an adapter or primer binding sequence at a target site, but is otherwise the same as the PEgRNA of interest. This RNP (i.e., PE complex) is incubated with extracted genomic DNA before or after fragmentation of the DNA. After fragmentation, different adapters sequences are ligated to the ends of the fragment DNA. PCR is used to amplify those genomic sites that span the inserted adapter sequence (i.e., inserted by EP) and the adapters ligated to fragment ends. High throughput sequencing between the adapters sequences can identify genomic sites of modification that are on-target and off-target. This *in vitro* editing method should enhance the sensitivity of detection because cellular DNA repair will not eliminate the reverse-transcribed DNA adapter added by the prime editor.

[0908] These methods could be used to identify off-target editing for any prime editor, or any genome editor that uses a guide RNA to recognize a target cut site (most Cas nucleases).

[0909] These methods could be applied to all genetic diseases for which genome editors are considered for use in treatment.

[0910] Exemplary adapter and/or primer binding sequences that may be installed by PE include, but are not limited to:

ADAPTER 1	5′-CGGTGGACCGATGATCT-3′ (SEQ ID NO: 177)
ADAPTER 2	5′-GCCACCTGGCTACTAGA-3′ (SEQ ID NO: 178)
ADAPTER 3	5′-AGATCATCGGTCCACCG-3′ (SEQ ID NO: 179)
ADAPTER 4	5′-TCTAGTAGCCAGGTGGC-3′ (SEQ ID NO: 180)

[0911] These adapter and/or primer binding sequences may also be used in the ligation step after genomic DNA fragmentation as outlined above.

[0912] Exemplary PEgRNA designs that illustrate the use of the herein described method to evaluate off-target editing, and their edit target locus, are as follows:

HEK3 TEST LOCUS OFF-TARGET DISCOVERY:	*GGCCCAGACTGAGCACGTGA*GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC**TCTGCCATCA***CGGTG GACCGATGATCT*CGTGCTCAGTCTG (SEQ ID NO: 181)
HEK4 TEST LOCUS OFF-TARGET DISCOVERY:	GGCACTGCGGCTGGAGGTGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC**ACACAGCACCAGAG TCTCCGCTTTAACCCCCA***GCCACCTGGCTACTAGA*CC TCCAGCC (SEQ ID NO: 182)
SICKLE CELL CORRECTION OFF-TARGET DISCOVERY:	GCATGGTGCACCTGACTCCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC**AGACTTCTCTTCA G***GCCACCTGGCTACTAGA*GAGTCAGGTGCAC (SEQ ID NO: 183)
KEY	*PBS Spacer* sgRNA scaffold **DNA synthesis template** *Adapter* PBS

K. Use of prime editing for insertion of inducible dimerization domains

[0913] The prime editors described herein may also be used to install dimerization domains into one or more protein targets. The dimerization domains may facilitate inducible regulation of the activity associated with the dimerization of the one or more protein targets via a linking moiety (e.g., a small molecule, peptide, or protein) that binds in a bi-specific manner. In various aspects, the dimerization domains, when installed on different proteins (e.g., the same type or different proteins), each bind to the same bi-specific moiety (e.g., a bi-

specific small molecule, peptide, or polypeptide having a least two regions that separately bind to the dimerization domains), thereby causing the dimerization of the proteins through their common interaction to the bi-specific ligand. In this manner, the bi-specific ligand functions as an "inducer" of dimerization of two proteins. In some cases, the bi-specific ligand or compound will have two targeting moieties that are the same. In other embodiments, the bi-specific ligand or compound will have targeting moieties that are each different from the other. The bi-specific ligand or compound having the same two targeting moieties will be able to target the same dimerization domain installed on different protein targets. The bi-specific ligand or compound having different targeting moieties will be able to target different dimerization domains installed on different protein targets.

[0914] As used herein, the term "dimerization domain" refers to a ligand-binding domain that binds to a binding moiety of a bi-specific ligand. A "first" dimerization domain binds to a first binding moiety of a bi-specific ligand and a "second" dimerization domain binds to a second binding moiety of the same bi-specific ligand. When the first dimerization domain is fused to a first protein (e.g., via PE, as discussed herein) and the second dimerization domain (e.g., via PE, as discussed herein) is fused to a second protein, the first and second protein dimerize in the presence of a bi-specific ligand, wherein the bi-specific ligand has at least one moeity that binds to the first dimerization domain and at least another moiety that binds to the second dimerization domain.

[0915] The term "bi-specific ligand" or "bi-specific moiety," as used herein, refers to a ligand that binds to two different ligand-binding domains. In various embodiments, the bi-specific moiety itself is a dimer of two of same or two different chemical moieties, wherein each moiety specifically and tightly binds to a dimerization domain. In certain embodiments, the ligand is a small molecule compound, or a peptide, or a polypeptide. In other embodiments, ligand-binding domain is a "dimerization domain," which can be install as a peptide tag onto a protein. In various embodiments, two proteins each comprising the same or different dimerization domains can be induced to dimerize through the binding of each dimerization domain to the bi-specific ligand. These molecules may also be referred to as "chemical inducers of dimerization" or CIDs. In addition, the bi-specific ligands may be prepared by coupling (e.g., through standardize chemical linkages) two of the same moieties together, or two different moieties together, wherein each moiety tightly and specifically binds to a dimerization domain.

[0916] In various aspects, the dimerization domains installed by PE can be the same or different.

[0917] For example, the dimerization domains can be FKBP12, which has the following amino acid sequence:

FKBP12	MGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKFDSSRDRNKPFKFMLGKQEVIR GWEEGVAQMSVGQRAKLTISPDYAYGATG HPGIIPPHATLVFDVELLKLE (SEQ ID NO: 488)

[0918] In another example, the dimerization domain can be a mutant of FKBP12 referred to as FKBP12-F36V, a mutant of FKBP12 with an engineered hole that binds a synthetic bumped FK506 mimic (2, Figure 3)[107]:

FKBP12-F36V	MGVQVETISPGDGRTFPKRGQTCVVHYTG MLEDGKKVDSSRDRNKPFKFMLGKQEVI RGWEEGVAQMSVGQRAKLTISPDYAYGAT GHPGIIPPHATLVFDVELLKLE (SEQ ID NO: 489)

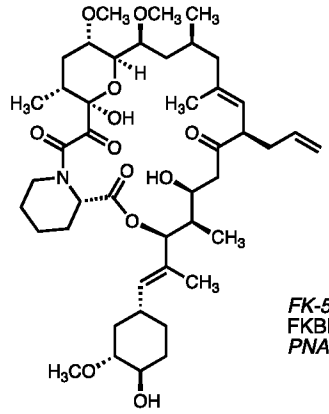
[0919] In another example, the dimerization domain can be cyclophilin, as follows:

CYCLOPHILIN	MVNPTVFFDIAVDGEPLGRVSFELFADKVP KTAENFRALSTGEKGFGYKGSCFHRIIPGF MCQGGDFTRHNGTGGKSIYGEKFEDENFI LKHTGPGILSMANAGPNTNGSQFFICTAKT EWLDGKHVVFGKVKEGMNIVEAMERFGS RNGKTSKKITIADCGQLE (SEQ ID NO: 490)

[0920] In various embodiments, the amino acid sequences of these dimerization domains may be altered in order to optimize binding or to improve binding orthogonality to native targets. The nucleic acid sequences of the genes encoding small-molecule binding proteins may be altered in order to optimize the efficiency of the PE process, such as by reducing PEgRNA secondary structure.

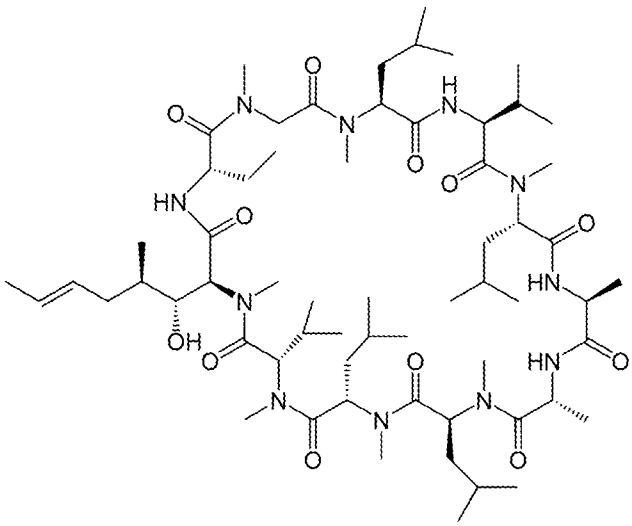
[0921] Other examples of suitable dimerization domains and a cognate small molecule compound which binds thereto are provided as follows. Note that the cognate small molecule compound could be coupled (e.g., via a chemical linker) to a second small molecule compound (either the same compound or a different compound) in order to form a bi-specific ligand that may bind two dimerization domains. In some cases, such as FK506 and cyclosporin A, dimerization of each (e.g., FK506-FK506 or cyclosporin A-cyclosporin A) reduces or eliminates immunosuppressive activity of the monomeric compounds.

SMALL MOLECULE – BINDS TO THE DIMERIZATION DOMAIN – A DIMER OF	DIMERIZATION DOMAIN(S)

<table>
<tr><td colspan="2">THESE MOLECULES WOULD CONSTITUTE A BI-SPECIFIC LIGAND THAT WOULD BIND TWO DIMERIZATION DOMAINS</td></tr>
<tr><td>FK506
 
FK-506
FKBP12 Kd 0.4 nM
PNAS 1990, 87, 9231.

TARGETS: FKBP12 + CALCINEURIN</td><td>AMINO ACID SEQUENCE OF FKBP12:

MGVQVETISPGDGRTFPKRGQTCVVH YTGMLEDGKKFDSSRDRNKPFKFMLG KQEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVELLKL E (SEQ ID NO: 491)

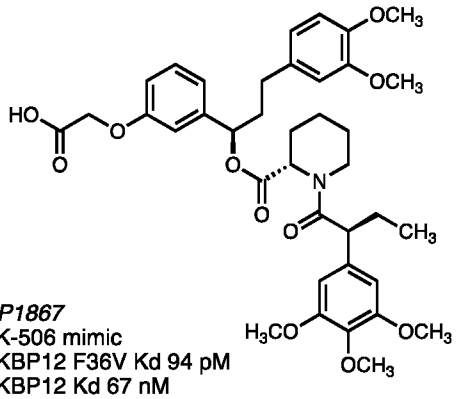
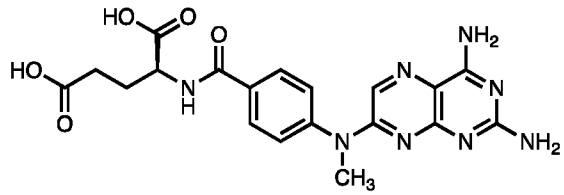
CALCINEURIN:</td></tr>
<tr><td>CYCLOSPORIN A
 
TARGETS: CYCLOPHILIN + CALCINEURIN</td><td>AMINO ACID SEQUENCE OF HUMAN CYCLOPHILIN A:

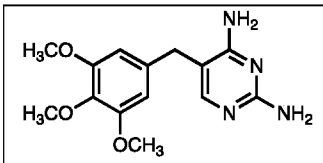
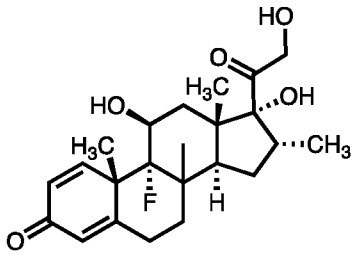
MVNPTVFFDIAVDGEPLGRVSFELF ADKVPKTAENFRALSTGEKGFGYK GSCFHRIIPGFMCQGGDFTRHNGTG GKSIYGEKFEDENFILKHTGPGILSM ANAGPNTNGSQFFICTAKTEWLDG KHVVFGKVKEGMNIVEAMERFGS RNGKTSKKITIADCGQLE (SEQ ID NO: 490)

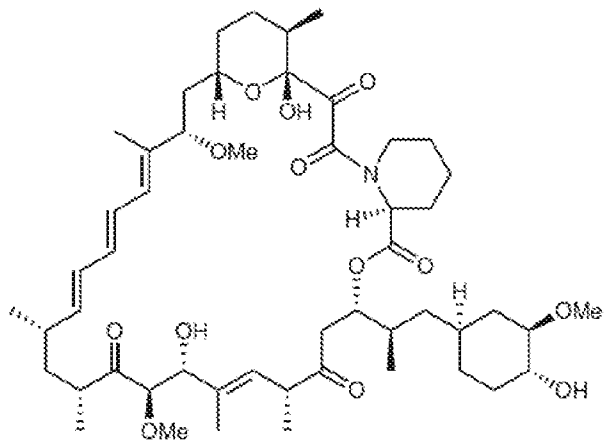
AMINO ACID SEQUENCE OF HUMAN CYCLOPHILIN B:

MLRLSERNMKVLLAAALIAGSVFF LLLPGPSAADEKKKGPKVTVKVYF DLRIGDEDVGRVIFGLFGKTVPKTV DNFVALATGEKGFGYKNSKFHRVIK DFMIQGGDFTRGDGTGGKSIYGER FPDENFKLKHYGPGWVSMANAGK DTNGSQFFITTVKTAWLDGKHVVF GKVLEGMEVVRKVESTKTDSRDKP LKDVIIADCGKIEVEKPFAIAKE (SEQ ID NO: 493)

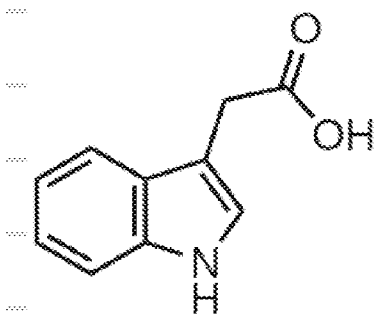
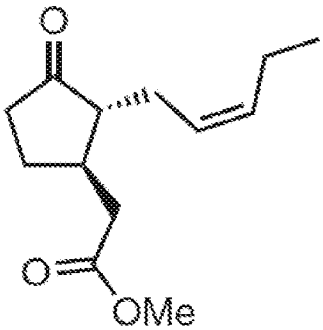
AMINO ACID SEQUENCE OF MURINE CYCLOPHILIN C:</td></tr>
</table>

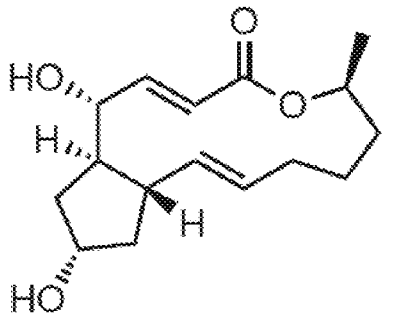
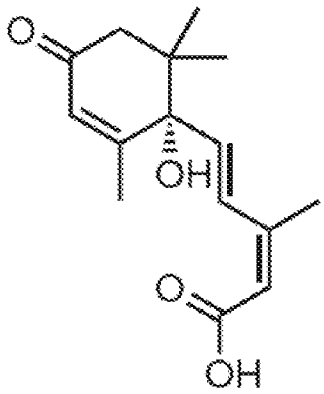
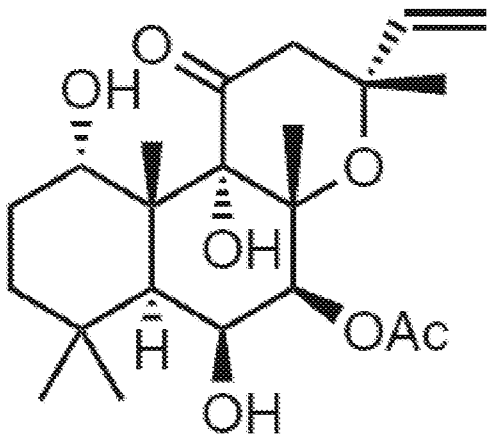
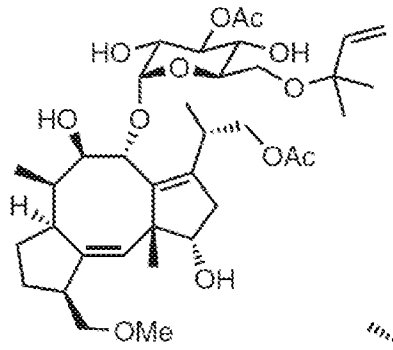
	MSPGPRLLLPAVLCLGLGALVSSSG SSGVRKRGPSVTDKVFFDVRIGDK DVGRIVIGLFGNVVPKTVENFVALA TGEKGYGYKGSIFHRVIKDFMIQGG DFTARDGTGGMSIYGETFPDENFKL KHYGIGWVSMANAGPDTNGSQFFI TLTKPTWLDGKHVVFGKVLDGMT VVHSIELQATDGHDRPLTDCTIVNS GKIDVKTPFVVEVPDW (SEQ ID NO: 494)
AP1867  *AP1867* FK-506 mimic FKBP12 F36V Kd 94 pM FKBP12 Kd 67 nM *PNAS* **1998**, *95*, 10437. TARGET(S): FKBP12	AMINO ACID SEQUENCE OF FKBP12-F36V MGVQVETISPGDGRTFPKRGQTCVVH YTGMLEDGKKVDSSRDRNKPFKFMLG KQEVIRGWEEGVAQMSVGQRAKLT ISPDYAYGATGHPGIIPPHATLVFDVELL KLE (SEQ ID NO: 495)
METHOTREXATE  *methotrexate* Human DHFR Kd < 10 nM *J. Biol. Chem.* **1988**, *263*, 10304. *E. coli* DHFR Kd 9.5 nM *PNAS* **2002**, *99*, 13481. TARGET(S): DIHYDROFOLATE REDUCTASE	AMINO ACID SEQUENCE OF HUMAN DIHYDROFOLATE REDUCTASE MVGSLNCIVAVSQNMGIGKNGDLPWP PLRNEFRYFQRMTTTSSVEGKQNLVIM GKKTWFSIPEKNRPLKGRINLVLSRELK EPPQGAHFLSRSLDDALKLTEQPELAN KVDMVWIVGGSSVYKEAMNHPGHLK LFVTRIMQDFESDTFFPEIDLEKYKLLP EYPGVLSDVQEEKGIKYKFEVYEKND (SEQ ID NO: 496)
TRIMETHOPRIM	AMINO ACID SEQUENCE FOR E. COLI DIHYDROFOLATE REDUCTASE MISLIAALAVDRVIGMENAMPWNLPAD LAWFKRNTLNKPVIMGRHTWESIGRPL PGRKNIILSSQPGTDDRVTWVKSVDEAI AACGDVPEIMVIGGGRVYEQFLPKAQ KLYLTHIDAEVEGDTHFPDYEPDDWES

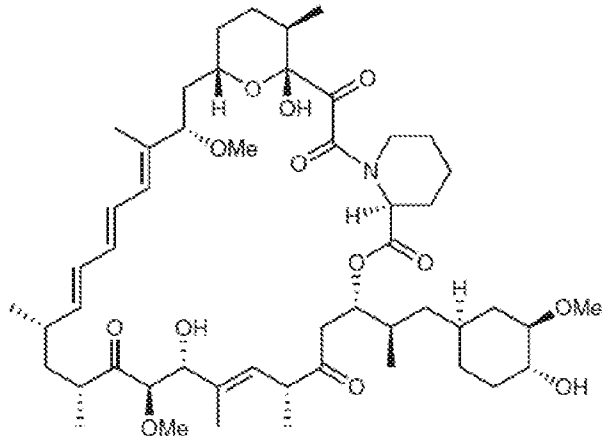
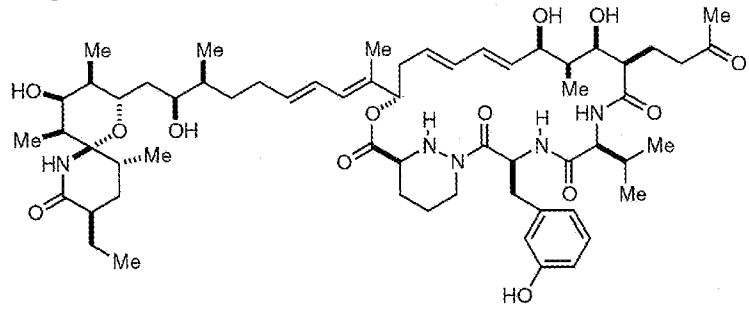
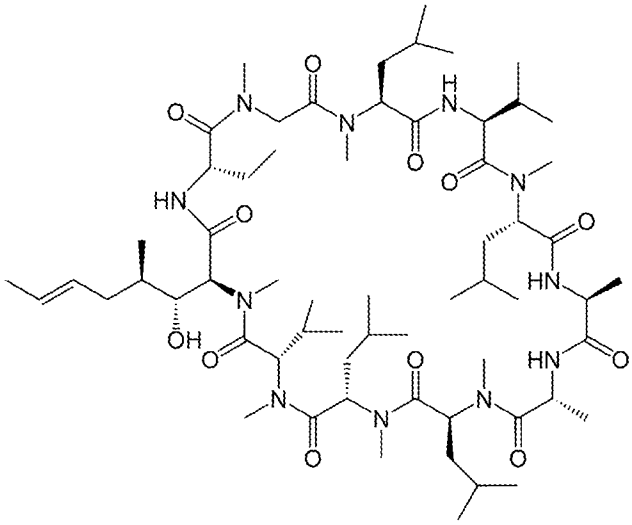
 *trimethoprim* *E. coli* DHFR K_I 1.3 nM *Biochemistry* **1982**, *21*, 5068. TARGET(S): DIHYDROFOLATE REDUCTASE	VFSEFHDADAQNSHSYCFEILERR (SEQ ID NO: 497)
DEXAMETHOSONE  *dexamethasone* Human GR Kd 4.6 nM *Mol. Endocrin.* **1999**, *13*, 1855.	AMINO ACID SEQUENCE FOR HUMAN GLUCOCORTICOID RECEPTOR MDSKESLTPGREENPSSVLAQERGDV MDFYKTLRGGATVKVSASSPSLAVASQ SDSKQRRLLVDFPKGSVSNAQQPDLSK AVSLSMGLYMGETETKVMGNDLGFPQ QGQISLSSGETDLKLLEESIANLNRSTS VPENPKSSASTAVSAAPTEKEF PKTHSDVSSEQQHLKGQTGTNGGNVK LYTTDQSTFDILQDLEFSSGSPGKETNE SPWRSDLLIDENCLLSPLAGEDDSFLLE GNSNEDCKPLILPDTKPKIKDNGDLVLS SPSNVTLPQVKTEKEDFIELCTPGVIKQ EKLGTVYCQASFPGANIIGNKMSAISV HGVSTSGGQMYHYDMNTASLSQQQD QKPIFNVIPPIPVGSENWNRCQGSGDD NLTSLGTLNFPGRTVFSNGYSSPSMRPD VSSPPSSSSTATTGPPPKLCLVCSDEASG CHYGVLTCGSCKVFFKRAVEGQHNYL CAGRNDCIIDKIRRKNCPACRYRKCLQ AGMNLEARKTKKKIKGIQQATTGVSQ ETSENPGNKTIVPATLPQLTPTLVSLLEV IEPEVLYAGYDSSVPDSTWRIMTTLNM LGGRQVIAAVKWAKAIPGFRNLHLDD QMTLLQYSWMFLMAFALGWRSYRQS SANLLCFAPDLIINEQRMTLPCMYDQC KHMLYVSSELHRLQVSYEEYLCMKTL LLLSSVPKDGLKSQELFDEIRMTYIKEL GKAIVKREGNSSQNWQRFYQLTKLLD SMHEVVENLLNYCFQTFLDKTMSIEFP EMLAEIITNQIPKYSNGNIKKLLFHQK (SEQ ID NO: 498)

 RAPAMYCIN	AMINO ACID SEQUENCE OF FKBP12: MGVQVETISPGDGRTFPKRGQTCVVH YTGMLEDGKKFDSSRDRNKPFKFMLG KQEVIRGWEEGVAQMSVGQRAKLTISP DYAYGATGHPGIIPPHATLVFDVELLKL E (SEQ ID NO: 491)

[0922] Other examples of naturally occurring bifunctional molecules and their dual target receptors are as follows. Prime editing may be used to install the dual target receptors into different proteins. Once the different proteins are modified by PE to contain a bifunctional molecule receptor, the bifunctional molecules may be introduced, thereby causing the dimerization of the proteins modified to comprise the different dimerization domains. Examples of pairings of (1) a biofunctional molecule and (2) their dual target receptors are as follows:

NATURALLY OCCURRING BIOFUNCTIONAL MOLECULES	TARGET RECEPTORS OF THE BIOFUNCTIONAL MOLECULE
 auxin	Target receptor 1: auxin receptor Target receptor 2: TIR1 E3 ligase
	Target receptor 1: JAZ receptor Target receptor 2: Col1 E3 ligase

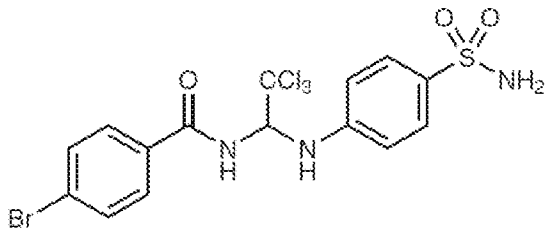
methyl jasmonate	
brefeldin A 	target receptor 1: GBF1 target receptor 2: GTPase Arf1p
abscisic acid 	target receptor 1: PYR receptor target receptor 2: phosphoprotein phosphatase 2C
Forskolin 	target receptor 1: adenylyl cyclase monomers target receptor 2: adenylyl cyclase monomers
fusicoccin A 	target receptor 1: 14-3-3 proteins target receptor 2: H^+-ATPase

Rapamycin 	target receptor 1: FKBP12 target receptor 2: mTOR
sanglifehrin A  Sanglifehrin A (SFA)	target receptor 1: cyclophilin target receptor 2: IMP dehydrogenase 2
cyclosporin A 	target receptor 1: cyclophilin target receptor 2: calcineurin

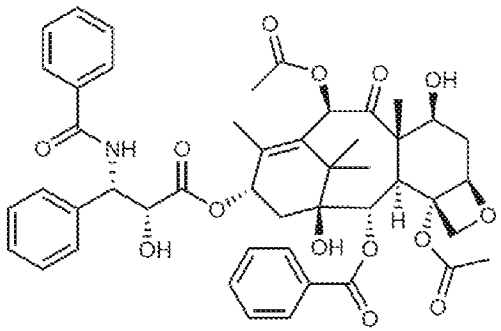
[0923]

[0924] Examples of other bifunctional molecules that can be used with this aspect of prime editing are as follows:

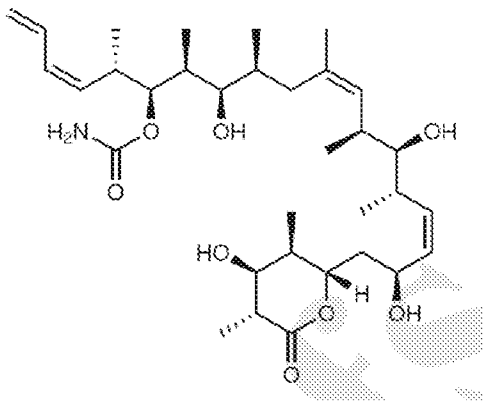
[0925] Synstab A:



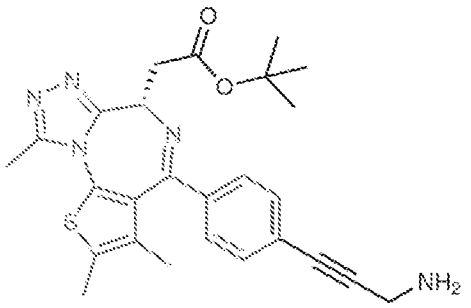
[0926] Paclitaxel:



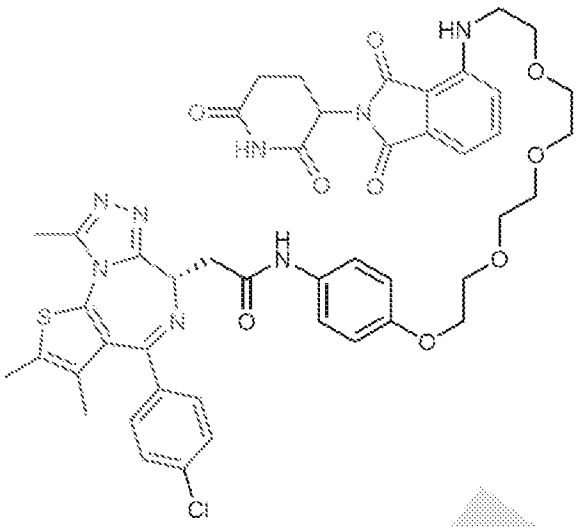
[0927] Discodermolide:



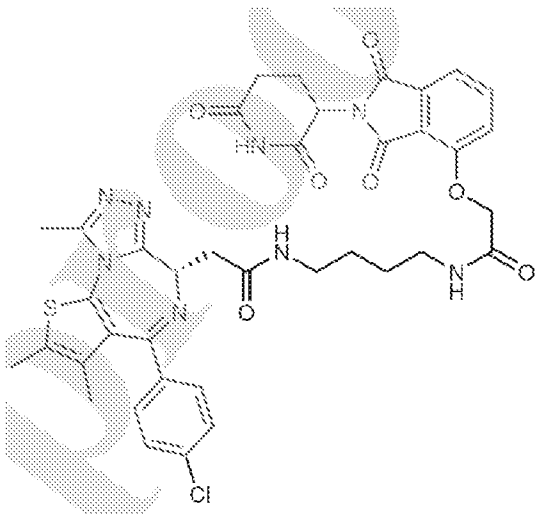
[0928] GNE-0011



[0929] ARV-825, and



[0930] dBET1



[0931] Synstab A, paclitaxel, and discodermolide are microtubule stabilizers. Thus, these compounds could be used to dimerize proteins modified by PE to comprise microtubule proteins. GNE-0011, ARV-825, and dBET1 comprise a BRD4 binding motif and a CRBN

binding motif. Thus, these compounds could be used to dimerize proteins modified by PE to comprise these targeting domains.

[0932] The PEgRNAs for installing dimerization domains may comprising the following structures (in reference to FIG. 3D):

5′-[spacer]-[gRNA core]-[extension arm]-3′, wherein the the extension arm comprises 5′-[homology arm]-[edit template]-[primer binding site]-3′; or

5′-[extension arm]-[spacer]-[gRNA core]-3′, wherein the the extension arm comprises 5′-[homology arm]-[edit template]-[primer binding site]-3′, and wherein with either configuration the "edit template" comprises a nucleotide sequence of a dimerization domain.

[0933] In one example, the PEgRNA for insertion of the FKBP12 dimerization domain at the C-terminal end of human insulin receptor (spacer underlined, gRNA core plain, flap homology bold, FKBP12 insertion in italics, annealing region bold italics):

PEGRNA FOR INSTALLING FKBP12 IN HUMAN INSULIN RECEPTOR	<u>CACGGUAGGCACUGUUAGGA</u>GUUUUAG AGCUAGAAAUAGCAAGUUAAAAUAAG GCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGC**UUGCCUCGGU** **CCAAUCCUUCC***GGAGUGCAGGUGGAAA* *CCAUCUCCCCAGGAGACGGGCGCACCUU* *CCCCAAGCGCGGCCAGACCUGCGUGGUG* *CACUACACCGGGAUGCUUGAAGAUGGAA* *AGAAAUUUGAUUCCUCCCGGGACAGAAA* *CAAGCCCUUUAAGUUUAUGCUAGGCAAG* *CAGGAGGUGAUCCGAGGCUGGGAAGAAG* *GGGUUGCCCAGAUGAGUGUGGGUCAGA* *GAGCCAAACUGACUAUAUCUCCAGAUUA* *UGCCUAUGGUGCCACUGGGCACCCAGGC* *AUCAUCCCACCACAUGCCACUCUCGUCU* *UCGAUGUGGAGCUUCUAAAACUGGAA****UA*** ***ACAGUGCCUACC*** (SEQ ID NO: 499)

[0934] In another example, the PEgRNA for insertion of the FKBP12 dimerization domain at the HEK3 locus (for optimization):

PEGRNA FOR INSTALLING FKBP12 IN HEK3	GGCCCAGACTGAGCACGTGAGTTTTAGA GCTAGAAATAGCAAGTTAAAATAAGGC TAGTCCGTTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTGGAGGAAGCAGGG CTTCCTTTCCTCTGCCATCATTCCAGTTT TAGAAGCTCCACATCGAAGACGAGAGT GGCATGTGGTGGGATGATGCCTGGGTGC CCAGTGGCACCATAGGCATAATCTGGAG ATATAGTCAGTTTGGCTCTCTGACCCAC

	ACTCATCTGGGCAACCCCTTCTTCCCAG CCTCGGATCACCTCCTGCTTGCCTAGCA TAAACTTAAAGGGCTTGTTTCTGTCCCG GGAGGAATCAAATTTCTTTCCATCTTCA AGCATCCCGGTGTAGTGCACCACGCAGG TCTGGCCGCGCTTGGGGAAGGTGCGCCC GTCTCCTGGGGAGATGGTTTCCACCTGC ACTCCCGTGCTCAGTCTG (SEQ ID NO: 500)

[0935] The target proteins for installing dimerization domains are not particularly limited; however, it is advantagous their dimerization (once modified by PE) in the presence of a bi-specific ligand produces some advantagous biological effect, e.g., a signaling pathway, decreased immunoresponsiveness, etc. In various aspects, the target proteins that are to be dimerized through the PE-dependent installation of dimerization domains can be the same protein or different proteins. Preferably, the proteins, when dimerized, trigger one or more downstream biological cascades, e.g., a signal transduction cascade, phorsphorylation, etc. Exemplary target proteins into which PE may be used to install dimerization domains, include, but are not limited to:

MEMBRANDE-BOUND RECEPTOR	KINASE DOMAIN FUSED TO	CID EMPLOYED	SIGNALING CASCADE	REFERENCE
T-CELL RECEPTOR	FKBP12	FK1012 (FK506 DIMER)	T-CELL RECEPTOR SIGNALING	*SCIENCE* 262, 1019–24 (1993) *CHEM. BIOL.* 1, 163–172 (1994).
FAS RECEPTOR	MURINE CYCLOPHILIN C	CYCLOSPORIN A DIMER	FAS PATHWAY FOR APOPTOSIS	*CHEM. BIOL.* 3, 731–738 (1996).
INSULIN RECEPTOR	FKBP12	FK1012 (FK506 DIMER)	INSULIN SIGNALING	*CURR. BIOL.* 8, 11–18 (1998).
PLATELET-DERIVED GROWTH FACTOR (PDGF) BETA	FKBP12	FK1012 (FK506 DIMER)	PDGF MESODERM FORMATION SIGNALING	*CURR. BIOL.* 8, 11–18 (1998).

ERYTHROPOIETIN RECEPTOR (EPOR)	FKBP12	FK1012 (FK506 DIMER)	EPOR-MEDIATED PROLIFERATIVE SIGNALING	*PROC. NATL. ACAD. SCI.* 94, 3076–3081 (2002).

[0936] In one aspect, prime editors described herein may be used to install sequences encoding dimerization domains into one or more genes encoding target proteins of interest in a living cell or patient. This may be referred to as the "prime editing–CID system," wherein the CID is the bi-specific ligand that induced dimerization of target proteins, each fused to a dimerization domain installed by PE. This edit alone should have no physiological effect. Upon administration of a bi-specific ligand, which typically is a dimeric small molecule that can simultaneously bind to two dimerization domaina each of which is fused to a copy of the target protein, the bi-specific ligand causes dimerization of the targeted protein. This target protein dimerization event then induces a biological signaling event, such as erythropoiesis or insulin signaling. A new method to place dimerization-induced biological processes, such as receptor signaling, under control of a convenient small-molecule drug (i.e., the bi-specific ligand) by the genomic integration of genes encoding small-molecule binding proteins (i.e., the dimerization domains) with prime editing is described herein.

[0937] Protein dimerization is a ubiquitous biological process. Notably, homodimerization of many membrane-bound receptors is known to initiate signaling cascades, often with profound biological consequences. A number of small-molecule natural products approved for use as drugs act as chemical inducers of protein dimerization as part of their mechanism of action.[92] For example, FK506 binds tightly to FKBP12, and the resulting small molecule–protein complex then binds the phosphatase calcineurin, thereby inhibiting a step in T cell receptor signaling.[93] Likewise, cyclosporin A induces dimerization of cyclophilin and calcineurin, and rapamycin induces dimerization of FKBP and mTOR.[93,94]

[0938] In one embodiment, leveraging the selective, high-affinity binding of the FK506:FKBP12 and cyclosporin A:cyclophilin small molecule:protein binding interaction, synthetic chemical inducers of dimerization have also been developed. In an example, a small molecule comprised of two units of FK506, termed FK1012, was shown to effect signal transduction when the cytoplasmic domains of signaling receptors were tagged with FKBP12.[95] Chemical inducers of dimerization (CIDs) have since been used to control a number of signaling pathways.[96-103]

[0939] While useful tools for studying biological processes, one challenge facing synthetic CIDs for therapeutic applications is that introduction of the FKBP12– or cyclophilin–target protein chimeras into patients is challenging.

[0940] The present disclosure brings together two concepts to create a previously inaccessible therapeutic process. The first concept is prime editing, described herein, which allows for precise genome editing, including targeted insertions, in living cells. The second concept is chemical-induced dimerization, a powerful tool that has enabled small-molecule control over signaling and oligomerization processes in cell culture.

[0941] Specific cases in which chemical control over protein dimerization may have had a beneficial therapeutic effect have been identified.

[0942] The insulin receptor is a heterotetrameric transmembrane protein that responds to insulin binding to the extracellular domain by phosphorylation of the cytoplasmic kinase domain.[104] An engineered chimeric protein composed of a membrane-localization component, the C-terminal kinase domain of the insulin receptor, and three copies of FKBP12 responds to FK1012 and initiates the insulin response in cell culture.[99] Similarly, it is expected that the fusion of FKBP12 to the C-terminal end of the kinase domain of the native insulin receptor in patient cells should allow for FK1012-dependent phosphorylation and initiation of the insulin signaling cascade. This system could replace or complement insulin use in patients who cannot make insulin (e.g., type-1 diabetics), or who respond weakly to insulin (e.g., type-2 diabetics).

[0943] Additionally, erythropoietin stimulates erythrocyte proliferation by binding to the erythropoietin receptor (EpoR), either inducing dimerization or a conformational change in a preformed receptor dimer which results in activation of the Jak/STAT signaling cascade.[105] It has been demonstrated that FK1012-induced oligomerization of the membrane-anchored cytoplasmic domain of EpoR tagged with FKBP12 is sufficient to initiate the signaling Jak/STAT signaling cascade and promote cell proliferation.[106] It is anticipated that fusing FKBP12 to native EpoR by prime editing in patient cells will allow for FK1012-induced control over erythrocyte proliferation (erythropoiesis). This system could be used to trigger red blood cell growth in anemic patients. FK1012-inducible EpoR could also be employed as an *in vivo* selectable marker for blood cells that have undergone *ex vivo* engineering.

[0944] In principle, any receptor tyrosine kinase could be viable target for a prime editing–CID therapeutic. The table below includes a list of all receptor tyrosine kinases in the human genome.[110]

Family	Receptor	Synonyms	NT Accession	PROT Accession	Chromosome
ALK family	ALK	CD	NM_004304	NP_004295	2p23
	LTK	TYK1	NM_002344	NP_002335	15q15.1-q21.1
AXL family	AXL	UFO, Tyro7(r) Ark(m)	NM_001699	NP_001690	19q13.1
	MER	MERTK, RPK, Eyk(m)	NM_006343	NP_006334	2q14.1
	TYRO3	RSE, SKY, BRT, DTK, TIF	NM_006293	NP_006284	15q15.1-q21.1
DDR family	DDR1	CAK, TRKE, NEP, NTRK4, EDDR1, PTK3	NM_013993	NP_001945	6p21.3
	DDR2	TKT, TYRO10, BTK6R3	NM_006182	NP_006173	1q21-q22
EGFR family	EGFR	ERBB, ERBB1	NM_005228	NP_005216	7p12
	ERBB2	HER2, Neu(r), NGL	NM_004448	NP_004439	17q11.2-q12
	ERBB3	HER3	NM_001982	NP_001973	12q13
	ERBB4	HER4	NM_005235	NP_005226	2q33.3-q34
EPH family	EPHA1	EPH, EPHT	NM_005232	NP_005223	7q32-q36
	EPHA2	ECK, Sek2(m), Myk2(m)	NM_004431	NP_004422	1p36
	EPHA3	HEK, ETK1, Tyro4(r), Mdk4(m), Cek4(ch)	NM_005233	NP_005224	3p11.2
	EPHA4	HEK8, Tyro1(r), Sek1(m), Cek8(ch)	NM_004438	NP_004429	2q36-ter
	EPHA5	HEK7, Ehk1(r), Bsk(r), Cek7(ch)	L36644	P54756	
	EPHA6	DKFZp434D1418, Ehk2(r)	AL133866		
	EPHA7	HEK11, Mdk1(m), Ebk(m), EPHX(m), Cek11(ch)	NM_004440	NP_004431	6q21
	EPHA8	HEK3, KIAA1445, Eek(r), Cek10(ch)	AB040892	CAB81612	1q23-q24
	EPHB1	NET, EPHT2, HEK6, Elk(r), Cek6(ch)	NM_004441	NP_004432	3q21-q23
	EPHB2	HEK5, ERK, DRT, EPHT3, Tyro5(r), Nuk(m), Sek3(m), Cek5(ch)	AF025304	AAB94802	1p36.1-p35
	EPHB3	HEK2, Tyro6, Mdk3(m), Sek4(m)	NM_004443	NP_004434	3q21-qter
	EPHB4	HTK, Tyro11(r), Mdk2(m), Myk1(m)	NM_004444	NP_004435	
	EPHB6	HEP, Mep(m), Cek1(ch)	NM_004445	NP_004436	7q33-q36
FGFR family	FGFR1	FLT2, bFGFR, FLG, N-SAM	M34641	AAA35835	8p11.2
	FGFR2	KGFR, K-SAM, Bek(m), CFD1, JWS, Cek3(ch)	NM_000141	NP_000132	10q26
	FGFR3	HSFGFR, ACH, Cek2(ch)	NM_000142	NP_000133	4p16.3
	FGFR4		NM_002011	NP_002002	5q35.1-qter
IGFR family	IGF1R	JTK13	NM_000875	NP_000866	15q25-q26
	INSR	IR	NM_000208	NP_000199	19p13.3-p13.2
	INSRR	IRR	J05046	AAC31759	1q21-q23
MET family	MET	HGFR	NM_000245	NP_000236	7q31
	RON	MST1R, CDw136, Pr2(m), STK(m), SEA(ch)	NM_002447	NP_002438	3p21.3
MUSK family	MUSK	Nsk2(m), Mdk1(m), Mtk2(m)	NM_005592	NP_005583	9q31.3-q32
PDGFR family	CSF1R	FMS, C-FMS, CD115	NM_005211	NP_005202	5q31-q32
	FLT3	FLK2, STK1, CD135	NM_004119	NP_004110	13q12
	KIT	SR(m), CKIT	NM_000222	NP_000213	4q11-q12
	PDGFRA		NM_006206	NP_006197	4q11-q13
	PDGFRB	PDGFR, JTK12	NM_002609	NP_002600	5q31-q32
PTK7 family	PTK7	CCK4, KLG(ch)	NM_002821	NP_002812	6p21.1-p12.2
RET family	RET	MEN2A/B, HSCR1, MTC1	X12949	P07949	10q11.2
ROR family	ROR1	NTRKR1	NM_005012	NP_005003	1p32-p31
	ROR2	NTRKR2	NM_004560	NP_004551	
ROS family	ROS1	MCF3	NM_002944	NP_002935	6q22
RYK family	RYK	Vik(m), Myk(m)	S69164	AAB25341	3q22
TIE family	TEK	TIE2	NM_000459	NP_000450	9p21
	TIE	TIE1, JTK14	NM_005424	NP_005415	1p34-p33
TRK family	NTRK1	TRK, TRKA	NM_002529	NP_002520	1q21-q22
	NTRK2	TRKB	NM_006180	NP_006171	9q22.1
	NTRK3	TRKC	NM_002530	NP_002521	15q25
VEGFR family	VEGFR1	FLT1	NM_002019	NP_002010	13q12
	VEGFR2	KDR, FLK1		AAB66626	4q11-q12
	VEGFR3	FLT4, PCL	NM_002020	NP_002011	5q34-q35
AATYK family	AATYK	AATK, KIAA0641	NM_004920	NP_004911	17q25.3
	AATYK2	KIAA1079	NM_014916	NP_055731	7q21-q22
	AATYK3				19p13.2-q13.3

[0945] There are numerous advantages to the prime editing–CID system. One such advantage is that it can replace endogenous ligands, which are typically proteins that pose complications in manufacturing, cost, delivery, production, or storage, with drug-like small-molecules that can be orally administered instead of administered by IV or injection, are readily prepared

from FDA-approved drugs (or are themselves already drugs), and do not incur special production or storage costs typically associated with protein drugs. Another advantage is that the edit alone should have no physiological effect. The amount of target protein dimerization can be controlled by dosing the small-molecule CID. Further, target protein dimerization is readily and rapidly reversible by adding the monomeric form of the CID. Yet another advantage is that in instances where a single ligand targets multiple receptors, selectivity can be achieved by prime-editing only one receptor. Finally, depending on the delivery method used for prime editing, it may also be possible to restrict editing to a localized tissue or organ, allowing for inducible receptor activation only in specific areas.

[0946] If editing efficiencies are high enough with prime editing that two separate editing events could occur at high levels, it would also be possible to tag two proteins of interest with different small-molecule binding domains (such as FKBP and cyclophilin) and induce heterodimerizations with small molecule heterodimers (such as an FK506–cyclosporin A dimer).

[0947] The fusion of FKBP12 or other small-molecule binding proteins to native proteins has been accomplished, generally by overexpression from plasmid in tissue culture. Subsequent chemical-induced dimerization has been demonstrated to induce phenotypic changes to cells producing the fusion proteins.

[0948] The following references are cited above in the Section G and are incorporated herein by reference.

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L. Use of prime editing for cell data recording

[0949] The prime editors and the resulting genomic modifications can also be used to study and record cellular processes and development. For example, the prime editors described herein may be used to record the presence and duration of a stimulus to a cell by providing to the cell a first nucleic acid sequence that encodes a fusion protein with a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase, and providing the cell at least a second nucleic acid sequence that encodes a PEgRNA. Either the first, the second, or both nucleic acid sequences are operablely linked to inducible promoters that are

responsive to the cell stimulus such that it induces expression of the fusion protein and/or the PEgRNA thereby causing the modification of a target sequence within the cell.

[0950] The prime editors described herein can also be used for cellular barcoding and lineage tracing. For example, by barcoding each cell with a unique genomic barcode, the prime editor can help reveal the cell lineage map by allowing the construction of phylogenetic trees based on the modifications made in one or more target sequence. Starting from progenitor cells, the prime editor system can enable building a cell-fate map for single cells in a whole organism, which can be deciphered by analyzing the modifications made in one or more target sequence. The method for tracing the linage of cells can include providing a nucleic acid encoding a fusion protein with a napDNAbp and a reverse transcriptase, and providing at least one second nucleic acid encoding a PEgRNA. A unique cellular barcode can be generated using the fusion protein and the PEgRNA to create one or more modifications in one or more target sequence, thereby allowing the linage of any cell that arises from the first cell to be traced using the unique cellular barcode. The use of prime editors for both cell data recording and linage tracing is further described in Example 13.

[0951] The prime editors can do perform both lineage tracing and cellular signaling recording by modifying genomic target sequences or integrated pre-designed sequences. Prime editors use a synthetic fusion protein comprising a Cas9 nickase fragment (including but not limited to the SpCas9 H840A variant) and a reverse transcriptase domain, along with an engineered prime editing guide RNA (PEgRNA). Together, these components target a specific genomic sequence or integrated pre-designed sequence and install a pre-determined edit. Because the PEgRNA specifies both the target genomic sequence and the edited outcome, highly specific and controlled genome modification can be achieved simultaneously using multiple PEgRNA within the same cell. Accessible genome modifications include all single nucleotide substitutions, small- to medium-sized sequence insertions, and small- to medium-sized deletions. The versatility of this genome editing technology can enable temporally coupled, signal-specific recording within cells.

[0952] The use of prime editors for cell data recording can include compositions (e.g., nucleic acids), cells, systems, kits, and methods for recording the strength and/or duration of endogenous or exogenous stimuli over the course of a cell's lifetime. The cell data recording system can include a fusion protein consisting of a napDNAbp (e.g., a Cas9 domain) and a reverse transcriptase operably linked to a promoter that induces the expression of the fusion protein to induce changes by creating targeted and sequence-specified genomic insertions,

deletions, or mutations in response to a stimulus or change in the cell. In contrast to digital memory devices that store information (e.g., the presence or absence of a stimulus) in one of two distinct states (i.e., "on" or "off"), these cell data recorders can induce permanent marks in cellular DNA in a manner that reflects both the strength (i.e., amplitude) and duration of one or more stimuli. Thus, in some aspects, cell data recording systems have the ability to simultaneously record multiple cell states, including, for example, exposure to a small molecule, a protein, a peptide, an amino acid, a metabolite, an inorganic molecule, an organometallic molecule, an organic molecule, a drug or drug candidate, a sugar, a lipid, a metal, a nucleic acid, a molecule produced during the activation of an endogenous or an exogenous signaling cascade, light, heat, sound, pressure, mechanical stress, shear stress, or a virus or other microorganism. These cell data recorders can employ sequencing technologies (e.g., high-throughput sequencing) to measure readout (e.g., changes in cellular DNA) and are not dependent on large cell populations for both the recording of a stimulus or the readout of the change(s) in cellular DNA induced by the stimulus.

[0953] In general, the cell data recorder systems provided herein for use in a cell comprise a fusion protein consisting of a napDNAbp and a reverse transcriptase, wherein the nucleic acid sequence encoding the fusion plasmid is operably linked to a promoter (e.g., an inducible promoter or a constitutive promoter). When a stimulus is present, or a change in cell state occurs, the stimulus induces the expression of the fusion protein. Also present within the cell are one or more nucleic acids encoding at least one PEgRNA that associate with the napDNAbp and directs the napDNAbp or the fusion protein to a target sequence (i.e., the PEgRNA is complementary to a target sequence). The nucleic acid encoding PEgRNA may also be, or may alternatively be, operably linked to a promoter (e.g., an inducible promoter or a constitutive promoter). Under the correct stimulus, or correct set of stimuli, both the fusion protein and the PEgRNA are expressed in the cell, and the PEgRNA associates with the fusion protein to direct it to a target sequence. This target sequence records the activity of the prime editor, thereby recording the presence of a stimulus, or a set of stimuli, or a change in cell state. More than one PEgRNA sequence can also be present in the cell, and these additional PEgRNA sequences, which can direct the fusion protein to distinct target sequences, can each be operably linked to a promoter that senses the presence of a different stimulus, allowing complex cell data recorder systems to be constructed for the ordered recording of the presence and duration of a stimulus, or set of stimuli. In some cases, one or more of the components of the cell data recorder system (e.g., fusion protein and PEgRNA)

may be constitutively expressed in the cell. Exemplary components of the cell data recorder system for use with the compositions are described herein. Additional suitable combinations of components provided herein will be apparent to a person of ordinary skill in the art based on this disclosure and knowledge in the field, and thus are embraced by the scope of this disclosure.

[0954] Repeated modification of a DNA target that can be sequenced by targeted amplicon sequencing and/or RNA sequencing (which is particular value for single cell recording experiments) can be used to record a host of important biological processes, including activation of signaling cascades, metabolic states, and cellular differentiation programs. Connecting internal and external cellular signals to sequence modification in the genome is possible for any signal for which a signal responsive promotor exists. In some embodiments, the promoter is a promoter suitable for use in a prokaryotic system (i.e., a bacterial promoter). In some embodiments, the promoter is a promoter suitable for use in a eukaryotic system (i.e., a eukaryotic promoter). In some embodiments, the promoter is a promoter suitable for use in a mammalian (e.g., human) system (i.e., a mammalian promoter). In some embodiments, the promoter is induced by a stimulus (i.e., an inducible promoter). In some embodiments, the stimulus is a small molecule, a protein, a peptide, an amino acid, a metabolite, an inorganic molecule, an organometallic molecule, an organic molecule, a drug or drug candidate, a sugar, a lipid, a metal, a nucleic acid, a molecule produced during the activation of an endogenous or an exogenous signaling cascade, light, heat, sound, pressure, mechanical stress, shear stress, or a virus or other microorganism, change in pH, or change in oxidation/reduction state. In some embodiments, the stimulus is a light. In some embodiments, the stimulus is a virus. In some embodiments, the stimulus is a small molecule. In some embodiments, the stimulus is an antibiotic. In some embodiments, the stimulus is anhydrotetracycline or doxycycline. In some embodiments, the stimulus is a sugar. In some embodiments, the stimulus is arabinose, rhamnose, or IPTG. In some embodiments, the stimulus is a signaling molecule produced during an activated signaling cascade (e.g., beta-catenin produced during an activated Wnt signaling cascade). Additional promoters that detect signaling molecules can be generated to induce the expression of the nucleic acid sequence operably linked to the promoter, for example, promoters that record an endogenous pathway, including immune response (IL-2 promoter), a cAMP responsive element (CREB), NFκB signaling, interferon response, P53 (DNA damage), Sox2, TGF-ß signaling (SMAD), Erk (e.g., from an activated Ras/Raf/Mek/Erk cascade), PI3K/AKT (e.g., from an activated

Ras/PI3K/Akt cascade), heat shock, Notch signaling, Oct4, an aryl hydrocarbon receptor, or an AP-1 transcription factor. In some embodiments, the promoter is a constitutive promoter. In some embodiments, the promoter is a promoter listed in Table 3. Additional suitable promoters for use in both prokaryotic and eukaryotic systems will be apparent to those of ordinary skill in the art based on this disclosure and knowledge in the field, and are within the scope of the present disclosure.

[0955] Prime editors can also be used to trace cellular lineages. Repeated sequence modifications can be used to generate unique cellular barcodes to track individual cells. The arrays of barcodes, their order, and size can all be used to infer cellular lineages. For example, the insertion of homology sequences (i.e., sequences 3′ of the Cas9 nick location), and in particular homology sequences with associated barcodes, appear to be particularly useful lineage prime editor strategies. These systems can be designed such that successive rounds of editing result in the insertion of a barcode from a PEgRNA cassette that cannot be modified by other PEgRNA editing events in the same cell. The barcoding system can utilize multiple barcodes that can be associated with a given stimulus. This system can preserve the majority of the target protospacers but alter the seed sequence, PAM, and downstream adjacent nucleotides. This enables multiple signals to be connected to one editing locus without significant re-designing of the PEgRNAs being used. The strategy can enable multiplexed barcode insertions in response to a large number of cellular stimuli (either internal or external) at a single locus. It could enable the recording of intensity, duration, and order of as many signals as there exist unique barcodes (which can be designed with multiple N nucleotides to generate 4^N possible barcodes, for example a 5-nt barcode would enable recording of 4^5 or 1024 unique signals at once). This system can be used both in vitro and in vivo.

M. Use of prime editing to modulate biomolecule activity

[0956] The use of prime editors described herein may also be used to regulate the subcellular localization and modification states of biomolecules, such as DNA, RNA, and proteins. Specific biological functions, like transcriptional control, cellular metabolism, and signal transduction cascades, are carefully orchestrated in particular locations within the cell. The ability to traffic proteins to these and other unique cellular compartments could provide an opportunity to alter a number of biological processes.

[0957] Accordingly, prime editing can be used to install genetically encoded handles that will allow for altered modification states and the subcellular trafficking of biomolecules with a

genetically encoded signal (e.g. proteins, lipids, sugars, and nucleic acids). In various embodiments, the target biomolecules for prime editor-mediated medication are DNA. For example, DNA could be modified by installing a number of DNA sequences that change the accessibility of the target locus, which could lead to either increased or decreased transcription of desired sequences. In other embodiments, the target biomolecules for prime editor-mediated medication are RNA. For example, the activity of RNA can be modified by changing its cellular localization, interacting partners, structural dynamics, or thermodynamics of folding. In yet other embodiments, the target biomolecules for prime editor-mediated medication are protein. Proteins can be modified to impact post-translational modifications, protein motifs can be installed to change the subcellular localization of the protein, or proteins can be modified to either create or destroy their ability to exist within protein-protein complexation events.

[0958] This application of prime editing can be further described in Example 14.

DNA modifications

[0959] One target biomolecule for PE-mediated modification is DNA. Modifications to DNA could be made to install a number of DNA sequences that change the accessibility of the target locus. Chromatin accessibility controls gene transcriptional output. Installation of marks to recruit chromatin compacting enzymes should decrease the transcriptional output of neighboring genes, while installation of sequences associated with chromatin opening should make regions more accessible and in turn increase transcription. Installation of more complex sequence motifs that mirror native regulatory sequences should provide more nuanced and biologically sensitive control than the currently available dCas9 fusions to different epigenetic reader, writer, or eraser enzymes–tools that typically install large numbers of a single type of mark that may not have a particular biological antecedent. Installation of sequences that will bring two loci into close proximity, or bring loci into contact with the nuclear membrane, should also alter the transcriptional output of those loci as has been demonstrated in the burgeoning field of 3-D genomic architecture.

RNA modifications

[0960] Modifications to RNAs can also be made to alter their activity by changing their cellular localization, interacting partners, structural dynamics, or thermodynamics of folding. Installation of motifs that will cause translational pausing or frameshifting could change the abundance of mRNA species through various mRNA processing mechanisms. Modifying consensus splice sequences would also alter the abundance and prevalence of different RNA

species. Changing the relative ratio of different splice isoforms would predictably lead to a change in the ratio of protein translation products, and this could be used to alter many biological pathways. For instance, shifting the balance of mitochondrial versus nuclear DNA repair proteins would alter the resilience of different cancers to chemotherapeutic reagents. Furthermore, RNAs could be modified with sequences that enable binding to novel protein targets. A number of RNA aptamers have been developed that bind with high affinity to cellular proteins. Installation of one of these aptamers could be used to either sequester different RNA species through binding to a protein target that will prevent their translation, biological activity, or to bring RNA species to specific subcellular compartments. Biomolecule degradation is another class of localization modification.

[0961] For example, RNA methylation is used to regulate RNAs within the cell. Consensus motifs for methylation could be introduced into target RNA coding sequences with PE. RNAs could also be modified to include sequences that direct nonsense mediated decay machinery or other nucleic acid metabolism pathways to degrade the target RNA species would change the pool of RNAs in a cell. Additionally, RNA species could be modified to alter their aggregation state. Sequences could be installed on single RNAs of interest or multiple RNAs to generate RNA tangles that would render them ineffective substrates for translation or signaling.

Protein modifications

[0962] Modifications to proteins via post-translational modification (PTM) also represent an important class of biomolecule manipulation that can be carried out with PE. As with RNA species, changing the abundance of proteins in a cell is an important capability of PE. Editing can be done to install stop codons in an open reading frame–this will eliminate full-length product from being produced by the edited DNA sequence. Alternatively, peptide motifs can be installed that cause the rate of protein degradation to be altered for a target protein. Installation of degradation tags into a gene body could be used to alter the abundance of a protein in a cell. Moreover, introduction of degrons that are induced by small molecules could enable temporal control over protein degradation. This could have important implications for both research and therapeutics as researchers could readily assess whether small molecule-mediated therapeutic protein degradation of a given target was a viable therapeutic strategy. Protein motifs could also be installed to change the subcellular localization of a protein. Amino acid motifs can be installed to preferentially traffic proteins

to a number of subcellular compartments including the nucleus, mitochondria, cell membrane, peroxisome, lysosome, proteasome, exosome, and others.

[0963] Installing or destroying motifs modified by PTM machinery can alter protein post-translational modifications. Phosphorylation, ubiquitylation, glycosylation, lipidation (e.g. farnesylation, myristoylation, palmitoylation, prenylation, GPI anchors), hydroxylation, methylation, acetylation, crotonylation, SUMOylation, disulfide bond formations, side chain bond cleavage events, polypeptide backbone cleavage events (proteolysis), and a number of other protein PTMs have been identified. These PTMs change protein function, often by changing subcellular localization. Indeed, kinases often activate downstream signaling cascades via phosphorylation events. Removal of the target phosphosite would prevent signal transduction. The ability to site-specifically ablate or install any PTM motif while retaining full-length protein expression would be an important advance for both basic research and therapeutics. The sequence installation scope and target window of PE make it well suited for broad PTM modification space.

[0964] Removal of lipidation sites should prevent the trafficking of proteins to cell membranes. A major limitation to current therapeutics that target post-translational modification processes is their specificity. Farnesyl transferase inhibitors have been tested extensively for their ability to eliminate KRAS localization at cell membranes. Unfortunately, global inhibition of farnesylation comes with numerous off target effects that have prevented broad use of these small molecules. Similarly, specific inhibition of protein kinases with small molecules can be very challenging due to the large size of the human genome and similarities between various kinases. PE offers a potential solution to this specificity problem, as it enables inhibition of modification of the target protein by ablation of the modification site instead of global enzyme inhibition. For example, removal of the lapidated peptide motif in KRAS would be a targeted approach that could be used in place of farnesyl transferase inhibition. This approach is the functional inverse of inhibiting a target protein activity by installing a lipid-targeting motif on a protein not designed to be membrane bound.

[0965] PE can also be used to instigate protein-protein complexation events. Proteins often function within complexes to execute their biological activity. PE can be used to either create or destroy the ability of proteins to exist within these complexes. To eliminate complex formation events, amino acid substitutions or insertions along the protein: protein interface could be installed to disfavor complexation. SSX18 is a protein component of the BAF complex, an important histone-remodeling complex. Mutations in SSX18 drive synovial

sarcomas. PE could be used to install side chains that prevent SSX18 from binding to its protein partners in the complex to prevent its oncogenic activity. PE could also be used to remove the pathogenic mutations to restore WT activity of this protein. Alternatively, PE could be used to keep proteins within either their native complex or to drag them to participate in interactions that are unrelated to their native activity to inhibit their activity. Forming complexes that maintain one interaction state over another could represent an important therapeutic modality. Altering protein: protein interfaces to decrease the Kd of the interaction would keep those proteins stuck to one another longer. As protein complexes can have multiple signaling complexes, like n-myc driving neuroblastoma signaling cascades in disease but otherwise participating in healthy transcriptional control in other cells. PE could be used to install mutations that drive n-myc association with healthy interactions partners and decrease its affinity for oncogenic interaction partners.

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N. Improved design aspects of PEgRNAs for prime editing

[0966] In other embodiments, the prime editing system may include the use of PEgRNA designs and strategies that can improve prime editing efficiency. These strategies seek to overcome some issues that exist because of the multi-step process required for prime editing. For example, unfavorable RNA structures that can form within the PEgRNA can result in the inhibition of DNA edits being copied from the PEgRNA into the genomic locus. These limitations could be overcome through the redesign and engineering of the PEgRNA component. These redesigns could improve prime editor efficiency, and could allow the installation of longer inserted sequences into the genome.

[0967] Accordingly, in various embodiments, the PEgRNA designs can result in longer PEgRNAs by enabling efficient expression of functional PEgRNAs from non-polymerase III (pol III) promoters, which would avoid the need for burdensome sequence requriements. In other embodiments, the core, Cas9-binding PEgRNA scaffold can be improved to improve efficacy of the system. In yet other embodiments, modifications can be made to the PEgRNA to improve reverse transcriptase (RT) processivity, which would enable the insertion of longer sequences at the targeted genomic loci. In other embodiments, RNA motifs can be added to the 5′ and/or 3′ termini of the PEgRNA to improve stability, enhance RT processivity, prevent misfolding of the PEgRNA, and/or recruit additional factors important for genome editing. In yet another embodiment, a platform is provided for the evolution of PEgRNAs for a given sequence target that could improve the PEgRNA scaffold and enhance prime editor efficiency. These designs could be used to improve any PEgRNA recognized by any Cas9 or evolved variant thereof.

[0968] This application of prime editing can be further described in Example 15.

[0969] The PEgRNAs may include additional design improvements that may modify the properties and/or characteristics of PEgRNAs thereby improving the efficacy of prime editing. In various embodiments, these improvements may belong to one or more of a number of different categories, including but not limited to: (1) designs to enable efficient expression of functional PEgRNAs from non-polymerase III (pol III) promoters, which would enable the expression of longer PEgRNAs without burdensome sequence requirements; (2) improvements to the core, Cas9-binding PEgRNA scaffold, which could improve efficacy; (3) modifications to the PEgRNA to improve RT processivity, enabling the insertion of longer sequences at targeted genomic loci; and (4) addition of RNA motifs to the 5′ or 3′ termini of the PEgRNA that improve PEgRNA stability, enhance RT processivity,

prevent misfolding of the PEgRNA, or recruit additional factors important for genome editing.

[0970] In one embodiment, PEgRNA could be designed with polIII promoters to improve the expression of longer-length PEgRNA with larger extension arms. sgRNAs are typically expressed from the U6 snRNA promoter. This promoter recruits pol III to express the associated RNA and is useful for expression of short RNAs that are retained within the nucleus. However, pol III is not highly processive and is unable to express RNAs longer than a few hundred nucleotides in length at the levels required for efficient genome editing. Additionally, pol III can stall or terminate at stretches of U's, potentially limiting the sequence diversity that could be inserted using a PEgRNA. Other promoters that recruit polymerase II (such as pCMV) or polymerase I (such as the U1 snRNA promoter) have been examined for their ability to express longer sgRNAs. However, these promoters are typically partially transcribed, which would result in extra sequence 5′ of the spacer in the expressed PEgRNA, which has been shown to result in markedly reduced Cas9:sgRNA activity in a site-dependent manner. Additionally, while pol III-transcribed PEgRNAs can simply terminate in a run of 6-7 U's, PEgRNAs transcribed from pol II or pol I would require a different termination signal. Often such signals also result in polyadenylation, which would result in undesired transport of the PEgRNA from the nucleus. Similarly, RNAs expressed from pol II promoters such as pCMV are typically 5′-capped, also resulting in their nuclear export.

[0971] Previously, Rinn and coworkers screened a variety of expression platforms for the production of long-noncoding RNA- (lncRNA) tagged sgRNAs[183]. These platforms include RNAs expressed from pCMV and that terminate in the ENE element from the MALAT1 ncRNA from humans[184], the PAN ENE element from KSHV[185], or the 3′ box from U1 snRNA[186]. Notably, the MALAT1 ncRNA and PAN ENEs form triple helices protecting the polyA-tail [184, 187]. These constructs could also enhance RNA stability. It is contemplated that these expression systems will also enable the expression of longer PEgRNAs.

[0972] In addition, a series of methods have been designed for the cleavage of the portion of the pol II promoter that would be transcribed as part of the PEgRNA, adding either a self-cleaving ribozyme such as the hammerhead[188], pistol[189], hatchet[189], hairpin[190], VS[191], twister[192], or twister sister[192] ribozymes, or other self-cleaving elements to process the transcribed guide, or a hairpin that is recognized by Csy4[193] and also leads to processing of the guide. Also, it is hypothesized that incorporation of multiple ENE motifs could lead to

improved PEgRNA expression and stability, as previously demonstrated for the KSHV PAN RNA and element[185]. It is also anticipated that circularizing the PEgRNA in the form of a circular intronic RNA (ciRNA) could also lead to enhanced RNA expression and stability, as well as nuclear localization[194].

[0973] In various embodiments, the PEgRNA may include various above elements, as exemplified by the following sequence.

[0974] Non-limiting example 1 - PEgRNA expression platform consisting of pCMV, Csy4 hairpin, the PEgRNA, and MALAT1 ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTAGGGTCATGAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTT
CTCAGGTTTTGCTTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGCAAAAGAT
GCTGGTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAATCCCTGCGGCGTCT
TTGCTTTGACT (SEQ ID NO: 501)

[0975] Non-limiting example 2 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and PAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC

ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAA (SEQ ID NO: 502)

[0976] Non-limiting example 3 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and 3xPAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC
ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAACACACTGT
TTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAG
GTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGC
CTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAA
GGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAATCTCTCTGTTTTGGCTGG
GTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAGGTTTTTATCC
CAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGCCTAGAGCTCA
AATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAAGGTCAATGTTT
AATCCATAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 503)

[0977] Non-limiting example 4 - PEgRNA expression platform consisting of pCMV, Csy4 hairing, the PEgRNA, and 3′ box

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTTGTTTGGTT
TGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 504)

[0978] Non-limiting example 5 - PEgRNA expression platform consisting of pU1, Csy4 hairping, the PEgRNA, and 3′ box

CTAAGGACCAGCTTCTTTGGGAGAGAACAGACGCAGGGGCGGGAGGGAAAAAG
GGAGAGGCAGACGTCACTTCCCCTTGGCGGCTCTGGCAGCAGATTGGTCGGTTGA
GTGGCAGAAAGGCAGACGGGGACTGGGCAAGGCACTGTCGGTGACATCACGGAC
AGGGCGACTTCTATGTAGATGAGGCAGCGCAGAGGCTGCTGCTTCGCCACTTGCT
GCTTCACCACGAAGGAGTTCCCGTGCCCTGGGAGCGGGTTCAGGACCGCTGATCG
GAAGTGAGAATCCCAGCTGTGTGTCAGGGCTGGAAAGGGCTCGGGAGTGCGCGG
GGCAAGTGACCGTGTGTGTAAAGAGTGAGGCGTATGAGGCTGTGTCGGGGCAGA
GGCCCAAGATCTCAGTTCACTGCCGTATAGGCAGGGCCCAGACTGAGCACGTGAG
TTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAGTCTGTTTCAGCAAG
TTCAGAGAAATCTGAACTTGCTGGATTTTTGGAGCAGGGAGATGGAATAGGAGCT
TGCTCCGTCCACTCCACGCATCGACCTGGTATTGCAGTACCTCCAGGAACGGTGC
ACCCACTTTCTGGAGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTT
GTTTGGTTTGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 505)

[0979] In various other embodiments, the PEgRNA may be improved by introducing improvements to the scaffold or core sequences. This can be done by introducing known

[0980] The core, Cas9-binding PEgRNA scaffold can likely be improved to enhance PE activity. Several such approaches have already been demonstrated. For instance, the first pairing element of the scaffold (P1) contains a GTTTT-AAAAC pairing element. Such runs of Ts have been shown to result in pol III pausing and premature termination of the RNA transcript. Rational mutation of one of the T-A pairs to a G-C pair in this portion of P1 has been shown to enhance sgRNA activity, suggesting this approach would also be feasible for PEgRNAs[195]. Additionally, increasing the length of P1 has also been shown to enhance sgRNA folding and lead to improved activity[195], suggesting it as another avenue for the improvement of PEgRNA activity. Example improvements to the core can include:

[0981] PEgRNA containing a 6 nt extension to P1

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGCTCATGAAAATGAGCTAGCAAG
TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTC
TGCCATCAAAGCGTGCTCAGTCTGTTTTTTT (SEQ ID NO: 228)

[0982] PEgRNA containing a T-A to G-C mutation within P1

GGCCCAGACTGAGCACGTGAGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGTTTTTTT (SEQ ID NO: 229)

[0983] In various other embodiments, the PEgRNA may be improved by introducing modifications to the edit template region. As the size of the insertion templated by the PEgRNA increases, it is more likely to be degraded by endonucleases, undergo spontaneous

hydrolysis, or fold into secondary structures unable to be reverse-transcribed by the RT or that disrupt folding of the PEgRNA scaffold and subsequent Cas9-RT binding. Accordingly, it is likely that modification to the template of the PEgRNA might be necessary to affect large insertions, such as the insertion of whole genes. Some strategies to do so include the incorporation of modified nucleotides within a synthetic or semi-synthetic PEgRNA that render the RNA more resistant to degradation or hydrolysis or less likely to adopt inhibitory secondary structures[196]. Such modifications could include 8-aza-7-deazaguanosine, which would reduce RNA secondary structure in G-rich sequences; locked-nucleic acids (LNA) that reduce degradation and enhance certain kinds of RNA secondary structure; 2'-O-methyl, 2'-fluoro, or 2'-O-methoxyethoxy modifications that enhance RNA stability. Such modifications could also be included elsewhere in the PEgRNA to enhance stability and activity. Alternatively or additionally, the template of the PEgRNA could be designed such that it both encodes for a desired protein product and is also more likely to adopt simple secondary structures that are able to be unfolded by the RT. Such simple structures would act as a thermodynamic sink, making it less likely that more complicated structures that would prevent reverse transcription would occur. Finally, one could also split the template into two, separate PEgRNAs. In such a design, a PE would be used to initiate transcription and also recruit a separate template RNA to the targeted site via an RNA-binding protein fused to Cas9 or an RNA recognition element on the PEgRNA itself such as the MS2 aptamer. The RT could either directly bind to this separate template RNA, or initiate reverse transcription on the original PEgRNA before swapping to the second template. Such an approach could enable long insertions by both preventing misfolding of the PEgRNA upon addition of the long template and also by not requiring dissociation of Cas9 from the genome for long insertions to occur, which could possibly be inhibiting PE-based long insertions.

[0984] In still other embodiments, the PEgRNA may be improved by introducing additional RNA motifs at the 5′ and 3′ termini of the PEgRNAs. Several such motifs - such as the PAN ENE from KSHV and the ENE from MALAT1 were discussed above as possible means to terminate expression of longer PEgRNAs from non-pol III promoters. These elements form RNA triple helices that engulf the polyA tail, resulting in their being retained within the nucleus[184,187]. However, by forming complex structures at the 3′ terminus of the PEgRNA that occlude the terminal nucleotide, these structures would also likely help prevent exonuclease-mediated degradation of PEgRNAs.

[0985] Other structural elements inserted at the 3′ terminus could also enhance RNA stability, albeit without enabling termination from non-pol III promoters. Such motifs could include hairpins or RNA quadruplexes that would occlude the 3′ terminus[197], or self-cleaving ribozymes such as HDV that would result in the formation of a 2'-3′-cyclic phosphate at the 3′ terminus and also potentially render the PEgRNA less likely to be degraded by exonucleases[198]. Inducing the PEgRNA to cyclize via incomplete splicing - to form a ciRNA - could also increase PEgRNA stability and result in the PEgRNA being retained within the nucleus[194].

[0986] Additional RNA motifs could also improve RT processivity or enhance PEgRNA activity by enhancing RT binding to the DNA-RNA duplex. Addition of the native sequence bound by the RT in its cognate retroviral genome could enhance RT activity[199]. This could include the native primer binding site (PBS), polypurine tract (PPT), or kissing loops involved in retroviral genome dimerization and initiation of transcription[199].

[0987] Addition of dimerization motifs - such as kissing loops or a GNRA tetraloop/tetraloop receptor pair[200] - at the 5′ and 3′ termini of the PEgRNA could also result in effective circularization of the PEgRNA, improving stability. Additionally, it is envisioned that addition of these motifs could enable the physical separation of the PEgRNA spacer and primer, prevention occlusion of the spacer which would hinder PE activity. Short 5′ or 3′ extensions to the PEgRNA that form a small toehold hairpin in the spacer region could also compete favorably against the annealing region of the PEgRNA binding the spacer. Finally, kissing loops could also be used to recruit other template RNAs to the genomic site and enable swapping of RT activity from one RNA to the other. Example improvements include, but are not limited to:

[0988] PEgRNA-HDV fusion

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAA
CATGCTTCGGCATGGCGAATGGGACTTTTTTT (SEQ ID NO: 230)

[0989] PEgRNA-MMLV kissing loop

GGTGGGAGACGTCCCACCGGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAA
TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTC
GGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACC
TTTTTTT (SEQ ID NO: 231)

[0990] PEgRNA-VS ribozyme kissing loop

GAGCAGCATGGCGTCGCTGCTCACGGCCCAGACTGAGCACGTGAGTTTTAGAGCT
AGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACC

GAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTCCATCAGTTGACA CCCTGAGGTTTTTTT (SEQ ID NO: 232)

[0991] PEgRNA-GNRA tetraloop/tetraloop receptor

GCAGACCTAAGTGGUGACATATGGTCTGGGCCCAGACTGAGCACGTGAGTTTTAG AGCTAUACGTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTUACGAAGTGG GACCGAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGCATGCGATT AGAAATAATCGCATGTTTTTTT (SEQ ID NO: 233)

[0992] PEgRNA template switching secondary RNA-HDV fusion

TCTGCCATCAAAGCTGCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACCGGCCG GCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG AATGGGACTTTTTTT (SEQ ID NO: 234)

[0993] PEgRNA scaffold could be further improved via directed evolution, in an analogous fashion to how SpCas9 and prime editor (PE) have been improved. Directed evolution could enhance PEgRNA recognition by Cas9 or evolved Cas9 variants. Additionally, it is likely that different PEgRNA scaffold sequences would be optimal at different genomic loci, either enhancing PE activity at the site in question, reducing off-target activities, or both. Finally, evolution of PEgRNA scaffolds to which other RNA motifs have been added would almost certainly improve the activity of the fused PEgRNA relative to the unevolved, fusion RNA. For instance, evolution of allosteric ribozymes composed of c-di-GMP-I aptamers and hammerhead ribozymes led to dramatically improved activity[202], suggesting that evolution would improve the activity of hammerhead-PEgRNA fusions as well. In addition, while Cas9 currently does not generally tolerate 5′ extension of the sgRNA, directed evolution will likely generate enabling mutations that mitigate this intolerance, allowing additional RNA motifs to be utilized.

[0994] The present disclosure contemplates any such ways to further improve the efficacy of the prime editing systems disclosed here.

O. Use of prime editing with expanded targeting scope

[0995] Prime editing (PE) using *Streptococcus pyogenes* Cas9 (SpCas9) can efficiently install all single base substitutions, insertions, deletions, and combinations thereof at genomic loci where there is a suitably-placed NGG protospacer adjacent motif (PAM) that SpCas9 can efficiently bind. However, in another asepct The methods described herein broaden the targeting capability of PE by expanding the accessible PAMs and, therefore, the targetable genomic loci accessible for efficient PE. Prime editors using RNA-guided DNA binding proteins other than SpCas9 enable an expanded targettable scope of genomic loci by allowing access to different PAMs. In addition, use of RNA-guided DNA binding proteins smaller

than SpCas9 also allows for more efficient viral delivery. PE with Cas proteins or other RNA-guided DNA binding proteins beyond SpCas9 will allow for high efficiency therapeutic edits that were either inaccessible or inefficient using SpCas9-based PE.

[0996] This is expected to be used in situations where SpCas9-based PE is either inefficient due to non-ideal spacing of an edit to relative to an NGG PAM or the overall size of the SpCas9-based construct is prohibitive for cellular expression and/or delivery. Specific disease-relevent loci such as the Huntingtin gene, which has few and poorly located NGG PAMs for SpCas9 near the target region, can easily be targeted using different Cas proteins in the PE system such as SpCas9-VRQR which recognizes an NGA PAM. Smaller Cas proteins will be used to generate smaller PE constructs that can be packaged into AAV vectors more efficiently, enabling better delivery to target tissues. FIG. 61 shows the reduction to practice of prime editing using *Staphylococcus aureus* CRISPR-Cas as the RNA-guided DNA binding protein. NT is untreated control.

[0997] FIGs. 62A-62B provide a demonstration of the importance of the protospacer for efficient installation of a desired edit at a precise location with prime editing. This highlights the importance of alternate PAMs and protospacers as novel features of this technology. "n.d." in FIG. 62A is "not detected."

[0998] FIG. 63 shows the reduction to practice of PE using SpCas9(H840A)-VRQR and SpCas9(H840A)-VRER as the RNA-guided DNA binding protein in a prime editor system. The SpCas9(H840A)-VRQR napDNAbp is disclosed herein as SEQ ID NO: 87. The SpCas9(H840A)-VRER napDNAbp is disclosed herein as SEQ ID NO: 88. The SpCas9(H840A)-VRER-MMLV RT fusion protein is disclosed herein as SEQ ID NO: 516, wherein the MMLV RT comprises the D200N, L603W, T330P, T306K, and W313F substitutions relative to the wild type MMLV RT. The SpCas9(H840A)-VRQR-MMLV RT fusion protein is disclosed herein as SEQ ID NO: 515, wherein the MMLV RT comprises the D200N, L603W, T330P, T306K, and W313F substitutions relative to the wild type MMLV RT. Seven different loci in the human genome are targeted: 4 with the SpCas9(H840A)-VRQR-MMLV RT prime editor system and 3 with the SpCas9(H840A)-VRER-MMLV RT system. The amino acid sequences of the tested contructs are as follows:

SACAS9-M-MLV RT PRIME EDITOR	MKRTADGSEFESPKKKRKVGKRNYILGLDIGITSVGYGIIDYETR DVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVKK LLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLA KRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLE RLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFID

	TYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPE ELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYH DIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQE EIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKL VPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYG LPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGK ENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII PRSVSFDNSFNNKVLVKQEEASKKGNRTPFQYLSSSDSKISYETF KKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVD TRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFK KERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMF EEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKK PNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLIN KSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGN YLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVK LSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYE EAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNL YEVKSKKHPQIIKKGSGGSSGGSSGSETPGTSESATPESSGGSSG GSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGL AVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGIL VPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVP NPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR DPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDL ILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQ ICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF LGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEI KQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRR PVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI LAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVV ALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHT WYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAEL IALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSE GKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRM ADQAARKAAITETPDTSTLLIENSSPSGGSKRTADGSEFEPKKKR KV (SEQ ID NO: 514)
SPCAS9(H840A)-VRQR-MALONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE PRIME EDITOR	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH

	SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 515)
SPCAS9(H840A)-VRER-MALONEY MURINE LEUKEMIA VIRUS REVERSE TRANSCRIPTASE PRIME EDITOR	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH

	SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKEYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 516)

[0999] As shown in FIG. 63, the SpCas9(H840A)-VRQR-MMLV RT was operational at PAM sites that included "AGAG" and "GGAG", with some editing activity at "GGAT" and "AGAT" PAM sequences. The SpCas9(H840A)-VRER-MMLV RT was operational at PAM sites that included "AGCG" and "GGCG", with some editing activity at "TGCG."

[1000] The data demonstrates that prime editing may be conducted using napDNAbps which bear different PAM specificities, such as those Cas9 variant described herein.

[1001] In various embodiments, the napDNAbp (e.g., Cas9) with altered PAM specificities

comprise a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAA-3′ PAM sequence at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 1. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 1. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 1.

[1002] Table 1: NAA PAM Clones

Mutations from wild-type SpCas9 (e.g., SEQ ID NO: 18)
D177N, K218R, D614N, D1135N, P1137S, E1219V, A1320V, A1323D, R1333K
D177N, K218R, D614N, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, G715C, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A367T, K710E, R1114G, D1135N, P1137S, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, D861N, D1135N, K1188R, E1219V, Q1221H, H1264H, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, V743I, R753G, M1021T, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, V743I, R753G, E762G, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, S1274R, A1320V, R1333K
A10T, I322V, S409I, E427G, A589S, R753G, D1135N, E1219V, Q1221H, H1264H, A1320V, R1333K
A10T, I322V, S409I, E427G, R753G, E757K, G865G, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, R753G, E757K, D1135N, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, K599R, M631A, R654L, K673E, V743I, R753G, N758H, E762G, D1135N, D1180G, E1219V, Q1221H, Q1256R, H1264Y, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N869S, N1054D, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, L727I, V743I, R753G, E762G, R859S, N946D, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, N1317T, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, K1151E, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, V1290G, L1318S, A1320V, A1323D, R1333K

A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, N803S, N869S, L921P, Y1016D, G1077D, F1080S, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, E630K, R654L, K673E, V743I, R753G, E762G, Q768H, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, Q768H, N803S, N869S, Y1016D, G1077D, R1114G, F1134L, D1135N, D1180G, E1219V, Q1221H, G1223S, H1264Y, L1318S, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, F693L, V743I, R753G, E762G, N803S, N869S, L921P, Y1016D, G1077D, F1801S, R1114G, D1135N, D1180G, E1219V, Q1221H, H1264Y, L1318S, A1320V, A1323D, R1333K
A10T, I322V, S409I, E427G, R654L, V743I, R753G, M1021T, D1135N, D1180G, K1211R, E1219V, Q1221H, H1264Y, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, M673I, N803S, N869S, G1077D, R1114G, D1135N, V1139A, D1180G, E1219V, Q1221H, A1320V, R1333K
A10T, I322V, S409I, E427G, R654L, K673E, V743I, R753G, E762G, N803S, N869S, R1114G, D1135N, E1219V, Q1221H, A1320V, R1333K

[1003] In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 1. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 1.

[1004] In some embodiments, the Cas9 protein exhibits an increased activity on a target sequence that does not comprise the canonical PAM (5′-NGG-3′) at its 3′ end as compared to *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18. In some embodiments, the Cas9 protein exhibits an activity on a target sequence having a 3′ end that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 5-fold increased as compared to the activity of *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18 on the same target sequence. In some embodiments, the Cas9 protein exhibits an activity on a target sequence that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold increased as compared to the activity of *Streptococcus pyogenes* as provided by SEQ ID NO: 2 on the same target sequence. In some embodiments, the 3′ end of the target sequence is directly adjacent to an AAA, GAA, CAA, or TAA sequence. In some embodiments, the Cas9 protein comprises a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAC-3′ PAM sequence

at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 2. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 2. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 2.

[1005] Table 2: NAC PAM Clones

MUTATIONS FROM WILD-TYPE SPCAS9 (E.G., SEQ ID NO: 18)
T472I, R753G, K890E, D1332N, R1335Q, T1337N
I1057S, D1135N, P1301S, R1335Q, T1337N
T472I, R753G, D1332N, R1335Q, T1337N
D1135N, E1219V, D1332N, R1335Q, T1337N
T472I, R753G, K890E, D1332N, R1335Q, T1337N
I1057S, D1135N, P1301S, R1335Q, T1337N
T472I, R753G, D1332N, R1335Q, T1337N
T472I, R753G, Q771H, D1332N, R1335Q, T1337N
E627K, T638P, K652T, R753G, N803S, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, K652T, R753G, N803S, K959N, R1114G, D1135N, K1156E, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, V647I, R753G, N803S, K959N, G1030R, I1055E, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, E630G, T638P, V647A, G687R, N767D, N803S, K959N, R1114G, D1135N, E1219V, D1332G, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, R1114G, D1135N, E1219V, N1266H, D1332N, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, I1057T, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, T638P, R753G, N803S, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
E627K, M631I, T638P, R753G, N803S, K959N, Y1036H, R1114G, D1135N, E1219V, D1251G, D1332G, R1335Q, T1337N
E627K, T638P, R753G, N803S, V875I, K959N, Y1016C, R1114G, D1135N, E1219V, D1251G, D1332G, R1335Q, T1337N, I1348V
K608R, E627K, T638P, V647I, R654L, R753G, N803S, T804A, K848N, V922A, K959N, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, R753G, N803S, V922A, K959N, K1014N, V1015A, R1114G, D1135N, K1156N, E1219V, N1252D, D1332N, R1335Q, T1337N
K608R, E627K, R629G, T638P, V647I, A711T, R753G, K775R, K789E, N803S, K959N, V1015A, Y1036H, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, T740A, R753G, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, T740A, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
I670S, K608R, E627K, E630G, T638P, V647I, R653K, R753G, I795L, K797N, N803S, K866R, K890N, K959N, Y1016C, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N

K608R, E627K, T638P, V647I, T740A, G752R, R753G, K797N, N803S, K948E, K959N, V1015A, Y1016S, R1114G, D1135N, E1219V, N1266H, D1332N, R1335Q, T1337N
I570T, A589V, K608R, E627K, T638P, V647I, R654L, Q716R, R753G, N803S, K948E, K959N, Y1016S, R1114G, D1135N, E1207G, E1219V, N1234D, D1332N, R1335Q, T1337N
K608R, E627K, R629G, T638P, V647I, R654L, Q740R, R753G, N803S, K959N, N990S, T995S, V1015A, Y1036D, R1114G, D1135N, E1207G, E1219V, N1234D, N1266H, D1332N, R1335Q, T1337N
I562F, V565D, I570T, K608R, L625S, E627K, T638P, V647I, R654I, G752R, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, N1177S, N1234D, D1332N, R1335Q, T1337N
I562F, I570T, K608R, E627K, T638P, V647I, R753G, E790A, N803S, K959N, V1015A, Y1036H, R1114G, D1135N, D1180E, A1184T, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, E627K, T638P, V647I, R654H, R753G, E790A, N803S, K959N, V1015A, R1114G, D1127A, D1135N, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, L625S, E627K, T638P, V647I, R654I, T703P, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
I570S, K608R, E627K, E630G, T638P, V647I, R653K, R753G, I795L, N803S, K866R, K890N, K959N, Y1016C, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N
I570T, K608R, E627K, T638P, V647I, R654H, R753G, E790A, N803S, K959N, V1016A, R1114G, D1135N, E1219V, K1246E, D1332N, R1335Q, T1337N
K608R, E627K, T638P, V647I, R654L, K673E, R753G, E790A, N803S, K948E, K959N, R1114G, D1127G, D1135N, D1180E, E1219V, N1286H, D1332N, R1335Q, T1337N
K608R, L625S, E627K, T638P, V647I, R654I, I670T, R753G, N803S, N808D, K959N, M1021L, R1114G, D1135N, E1219V, N1286H, D1332N, R1335Q, T1337N
E627K, M631V, T638P, V647I, K710E, R753G, N803S, N808D, K948E, M1021L, R1114G, D1135N, E1219V, D1332N, R1335Q, T1337N, S1338T, H1349R

[1006] In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 80% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 2. In some embodiments, the Cas9 protein comprises an amino acid sequence that is at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a Cas9 protein as provided by any one of the variants of Table 2.

[1007] In some embodiments, the Cas9 protein exhibits an increased activity on a target sequence that does not comprise the canonical PAM (5′-NGG-3′) at its 3′ end as compared to *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18. In some embodiments, the Cas9 protein exhibits an activity on a target sequence having a 3′ end that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 5-fold increased as compared to the activity of *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 18 on the same target sequence. In some embodiments, the Cas9 protein exhibits an activity on a target sequence that is not directly adjacent to the canonical PAM sequence (5′-NGG-3′) that is at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least

500,000-fold, or at least 1,000,000-fold increased as compared to the activity of *Streptococcus pyogenes* as provided by SEQ ID NO: 18 on the same target sequence. In some embodiments, the 3′ end of the target sequence is directly adjacent to an AAC, GAC, CAC, or TAC sequence.

[1008] In some embodiments, the Cas9 protein comprises a combination of mutations that exhibit activity on a target sequence comprising a 5′-NAT-3′ PAM sequence at its 3′-end. In some embodiments, the combination of mutations are present in any one of the clones listed in Table 3. In some embodiments, the combination of mutations are conservative mutations of the clones listed in Table 3. In some embodiments, the Cas9 protein comprises the combination of mutations of any one of the Cas9 clones listed in Table 3.

[1009] Table 3: NAT PAM Clones

MUTATIONS FROM WILD-TYPE SPCAS9 (E.G., SEQ ID NO: 18)
K961E, H985Y, D1135N, K1191N, E1219V, Q1221H, A1320A, P1321S, R1335L
D1135N, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
V743I, R753G, E790A, D1135N, G1218S, E1219V, Q1221H, A1227V, P1249S, N1286K, A1293T, P1321S, D1322G, R1335L, T1339I
F575S, M631L, R654L, V748I, V743I, R753G, D853E, V922A, R1114G D1135N, G1218S, E1219V, Q1221H, A1227V, P1249S, N1286K, A1293T, P1321S, D1322G, R1335L, T1339I
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, N1286K, P1321S, D1322G, R1335L
M631L, R654L, R753G, K797E, D853E, V922A, D1012A, R1114G D1135N, G1218S, E1219V, Q1221H, P1249S, N1317K, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, D596Y, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, Q1256R, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, K710E, V750A, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, K649R, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, K1156E, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1322G, R1335L
F575S, M631L, R654L, R664K, R753G, D853E, V922A, I1057G, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, N1308D, P1321S, D1322G, R1335L
M631L, R654L, R753G, D853E, V922A, R1114G, Y1131C, D1135N, E1150V, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L
M631L, R654L, R664K, R753G, D853E, I1057V, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L
M631L, R654L, R664K, R753G, I1057V, R1114G, Y1131C, D1135N, D1180G, G1218S, E1219V, Q1221H, P1249S, P1321S, D1332G, R1335L

[1010] Any of the above Cas9 variants displaying differential PAM specificities as compared to the canonical SpCas9 may be used in the herein disclosed prime editors.

P. Use of prime editing for inserting recombinase target sites

[1011] In another aspect, prime editing may be used to insert recombinase sites (or "recombinase recognition sequences") into a desired genomic site. Insertion of recombinase sites provides a programmed location for effecting site-specific genetic changes in a genome. Such genetic changes can include, for example, genomic integration of a plasmid, genomic deletion or insertion, chromosomal translocations, and cassette exchanges, among other genetic changes. These exemplary types of genetic changes are illustrated in FIG. 64(b)-(f). The installed recombinase recognition sequences may then be used to conduct site-specific recombination at that site to effecuate a variety of recombination outcomes, such as, excision, integration, inversion, or exhange of DNA fragments. For example, FIG. 65 illustrates the installation of a recombinase site that can then be used to integrate a DNA donor template comprising a GFP expression marker. Cells containing the integrated GFP expression system into the recombinase site will fluoresce.

[1012] The mechanism of installing a recombinase site into the genome is analogous to installing other sequences, such as peptide/protein and RNA tags, into the genome. A schematic exemplifying the installation of a recombinase target sequence is shows in FIG. 64(a). The process begins with selecting a desired target locus into which the recombinase target sequence will be introduced. Next, a prime editor fusion is provided ("RT-Cas9:gRNA"). Here, the "gRNA" refers to a PEgRNA, which can be designed using the principles described herein. The PEgRNA in various embodiments will comprise an architecture corresponding to FIG. 3D (5′-[~20-nt spacer]-[gRNA core]-[extension arm]-3′, wherein the extension arm comprises in the 3′ to 5′ direction, a primer binding site ("A"), an edit template ("B"), and a homology arm ("C"). The edit template ("B") will comprise a sequence corresponding to a recombinase site, i.e., a single strand RNA of the PEgRNA that codes for a complementary single strand DNA that is either the sense or the antisense strand of the recombinase site and which is incorporated into the genomic DNA target locus through the prime editing process.

[1013] In various aspects, the present dislosure provides for the use of a PE to introduce recombinase recognition sequences at high-value loci in human or other genomes, which, after exposure to site-specific recombinase(s), will direct precise and efficient genomic modifications (FIG. 64). In various embodiments show in FIG. 64, PE may be used to (b)

insert a single SSR target for use as a site for genomic integration of a DNA donor template. (c) shows how a tandem insertion of SSR target sites can be used to delete a portion of the genome. (d) shows how a tandem insertion of SSR target sites can be used to invert a portion of the genome. (e) shows how the insertion of two SSR target sites at two distal chromosomal regions can result in chromosomal translocation. (f) shows how the insertion of two different SSR target sites in the genome can be used to exchange a cassette from a DNA donor template. Each of the types of genome modifications are envisioned by using PE to insert SSR tagets, but this list also is not meant to be limiting.

[1014] PE-mediated introduction of recombinase recognition sequences could be particularly useful for the treatment of genetic diseases which are caused by large-scale genomic defects, such as gene loss, inversion, or duplication, or chromosomal translocation[1-7] (Table 6). For example, Williams-Beuren syndrome is a developmental disorder caused by a deletion of 24 in chromosome 721. No technology exists currently for the efficient and targeted insertion of multiple entire genes in living cells (the potential of PE to do such a full-length gene insertion is currently being explored but has not yet been established); however, recombinase-mediated integration at a target inserted by PE offers one approach towards a permanent cure for this and other diseases. In addition, targeted introduction of recombinase recognition sequences could be highly enabling for applications including generation of transgenic plants, animal research models, bioproduction cell lines, or other custom eukaryotic cell lines. For example, recombinase-mediated genomic rearrangement in transgenic plants at PE-specific targets could overcome one of the bottlenecks to generating agricultural crops with improved properties[8,9].

[1015] Table 6. Examples of genetic diseases linked to large-scale genomic modifications that could be repaired through PE-based installation of recombinase recognition sequences.

DISEASE	CAUSE
TRISOMY 17P	GENE DUPLICATION
CHARCOT-MARIE-TOOTH DISEASE TYPE I	GENE DUPLICATION
SMITH-MAGENIS SYNDROME	GENE DELETION
WILLIAMS-BEUREN SYNDROME	GENE DELETION
DE LA CHAPELLE SYNDROME	CHROMOSOMAL TRANSLOCATION
DOWN SYNDROME (SOME FORMS)	CHROMOSOMAL TRANSLOCATION
HEMOPHILIA A	GENE INVERSION

HUNTER SYNDROME	GENE INVERSION

[1016] A number of SSR family members have been characterized and their recombinase recognition sequences described, including natural and engineered tyrosine recombinases (Table 7), large serine integrases (Table 8), serine resolvases (Table 9), and tyrosine integrases (Table 10). Modified target sequences that demonstrate enhanced rates of genomic integration have also been described for several SSRs[22-30]. In addition to natural recombinases, programmable recombinases with distinct specificities have been developed[31-40]. Using PE, one or more of these recognition sequences could be introduced into the genomic at a specified location, such as a safe harbor locus[41-43], depending on the desired application.

[1017] For example, introduction of a single recombinase recognition sequence in the genome by prime editing would result in integrative recombination with a DNA donor template (FIG. 64b). Serine integrases, which operate robustly in human cells, may be especially well-suited for gene integration[44,45].

[1018] Additionally, introduction of two recombinase recognition sequences could result in deletion of the intervening sequence, inversion of the intervening sequence, chromosomal translocation, or cassette exchange, depending on the identity and orientation of the targets (FIG. 64c-f). By choosing endogenous sequences that already closely resemble recombinase targets, the scope of editing required to introduce the complete recombinase target would be reduced.

[1019] Finally, several recombinases have been demonstrated to integrate into human or eukaryotic genomes at natively occurring pseudosites[46-64]. PE editing could be used to modify these loci to enhance rates of integration at these natural pseudosites, or alternatively, to eliminate pseudosites that may serve as unwanted off-target sequences.

[1020] This disclosure describes a general methodology for introducing recombinase target sequences in eukaryotic genomes using PE, the applications of which are nearly limitless. The genome editing reactions are intended for use with "prime editor," a chimeric fusion of a CRISPR/Cas9 protein and a reverse-transcriptase domain, which utilizes a custom prime editing guide RNA (PEgRNA). By extension, Cas9 tools and homology-directed repair (HDR) pathways may also be exploited to introduce recombinase recognition sequences through DNA templates by lowering the rates of indels using several techniques[65-67]. A proof-of-concept experiment in human cell culture is shown in FIG. 65.

[1021] The following several tables are cited in the above description relating to PE-directed installation of recombinase recognition sequences and provide a listing of exemplary recombinases that may be used, and their cognate recombinase recognition sequences that may be installed by PE.

Table 7. Tyrosine recombinases and SSR target sequences.

Recombinase	Recombinase recognition sequence	Name
Cre	ATAACTTCGTATAGCATACATTATACGAAGTTAT (SEQ ID NO: 517)	*loxP*
Dre	TAACTTTAAATAATGCCAATTATTTAAAGTTA (SEQ ID NO: 518)	*rox*
VCre	TCAATTTCTGAGAACTGTCATTCTCGGAAATTGA (SEQ ID NO: 519)	*loxV*
SCre	CTCGTGTCCGATAACTGTAATTATCGGACATGAT (SEQ ID NO: 520)	*loxS*
Flp	GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC (SEQ ID NO: 521)	*FRT*
B2	GAGTTTCATTAAGGAATAACTAATTCCCTAATGAA ACTC (SEQ ID NO: 522)	*loxB*
B3	GGTTGCTTAAGAATAAGTAATTCTTAAGCAACC (SEQ ID NO: 523)	*loxB3*
Kw	ACGAAAAATGGTAAGGAATAGACCATTCCTTACC ATTTTTGGT (SEQ ID NO: 524)	
R	TTGATGAAAGAATAACGTATTCTTTCATCAA (SEQ ID NO: 525)	*RS*
TD1-40	GTGCGTCAAATAATAACGTATTATTTGACACTT (SEQ ID NO: 526)	*TDRS*
Vika	AATAGGTCTGAGAACGCCCATTCTCAGACGTATT (SEQ ID NO: 527)	*vox*
Nigri	TGAATGTCCTATAATTACACTTATAGGACATTCA (SEQ ID NO: 528)	*nox*
Panto	GAAACTTTAAATAATAAGTCTTATTTAAAGTTTC (SEQ ID NO: 529)	*pox*
Kd	AAACGATATCAGACATTTGTCTGATAATGCTTCA TTATCAGACAAATGTCTGATATCGTTT (SEQ ID NO: 530)	*loxK*
Fre	ATATATACGTATATAGACATATATACGTATATAT (SEQ ID NO: 531)	*loxH*
CreALSHG	ATAACTCTATATAATGTATGCTATATAGAGTTAT (SEQ ID NO: 532)	*loxM7*

Recombinase	Recombinase recognition sequence	Name
Tre	ACAACATCCTATTACACCCTATATGCCAACATGG (SEQ ID NO: 533)	*loxLTR*
Brec1	AACCCACTGCTTAAGCCTCAATAAAGCTTGCCTT (SEQ ID NO: 534)	*loxBTR*
Cre-R3M3	GATACAACGTATATACCTTTCTATACGTTGTTTA (SEQ ID NO: 535)	*loxK2*

Table 8. Large serine integrases and SSR target sequences.

Recombinase	Recombinase recognition sequence Left	Recombinase recognition sequence Right
Bxb1	GGTTTGTCTGGTCAACCACCGC GGTCTCAGTGGTGTACGGTACA AACC (SEQ ID NO: 536)	GGCTTGTCGACGACGGCGGTCTCC GTCGTCAGGATCAT (SEQ ID NO: 537)
phiC31	GTGCCCCAACTGGGGTAACCTT TGAGTTCTCTCAGTTGGGGG (SEQ ID NO: 538)	TGCGGGTGCCAGGGCGTGCCCTTG GGCTCCCCGGGCGCGTACTCC (SEQ ID NO: 539)
R4	TGTTCCCCAAAGCGATACCACT TGAAGCAGTGGTACTGCTTGTG GGTACA (SEQ ID NO: 540)	GCATGTTCCCCAAAGCGATACCAC TTGAAGCAGTGGTACTGCTTGTGG GTACACTCTGCGGGTG (SEQ ID NO: 541)
phiBT1	GGTGCTGGGTTGTTGTCTCTGG ACAGTGATCCATGGGAAACTA CTCAGCACC (SEQ ID NO: 542)	CAGGTTTTTGACGAAAGTGATCCA GATGATCCAG (SEQ ID NO: 543)
MJ1 (phiFC1)	ATTTTAGGTATATGATTTTGTT TATTAGTGTAAATAACACTATG TACCTAAAAT (SEQ ID NO: 544)	CAAAGGATCACTGAATCAAAAGTA TTGCTCATCCACGCGAAA (SEQ ID NO: 545)
MR11	TTTGTGCGGAACTACGAACAGT TCATTAATACGAAGTGTACAA ACTTCCATACAA (SEQ ID NO: 546)	CGAAAATGTATGGAGGCACTTGTA TCAATATAGGATGTATACCTTCGA AGACACTT (SEQ ID NO: 547)
TP901-1	GAGTTTTTATTTCGTTTATTTCA ATTAAGGTAACTAAAAAACTC CTTTTAAGG (SEQ ID NO: 548)	ATGCCAACACAATTAACATCTCAA TCAAGGTAAATGCTTTTTGCTTTTT TTGC (SEQ ID NO: 549)
A118	TTCCTCGTTTTCTCTCGTTGGA AGAAGAAGAAACGAGAAA (SEQ ID NO: 550)	TTTCGGATCAAGCTATGAAGGACG CAAAGAGGGAACTAAA(SEQ ID NO: 551)
U153	TTCCTCGTTTTCTCTCGTTGGAC GGAAACGAATCGAGAAA (SEQ ID NO: 552)	TTTCGGATCAAGCTATGAAGGACG CAAAGAGGGAACTAAA (SEQ ID NO: 553)
phiRV1	GTAGTGTATCTCACAGGTCCAC GGTTGGCCGTGGACTGCTGAA	GAAGGTGTTGGTGCGGGGTTGGCC GTGGTCGAGGTGGGGT (SEQ ID NO:

Recombinase	Recombinase recognition sequence Left	Recombinase recognition sequence Right
	GAACATTCC (SEQ ID NO: 554)	555)
phi370.1	AAAAAAATACAGCGTTTTTCATGTACAACTATACTAGTTGTAGTGCCTAAAA (SEQ ID NO: 556)	TTGTAAAGGAGACTGATAATGGCATGTACAACTATACTCGTCGGTAAAAAGGCA (SEQ ID NO: 557)
TG1	TCCAGCCCAACAGTGTTAGTCTTTGCTCTTACCCAGTTGGGCGGGA (SEQ ID NO: 558)	GATCAGCTCCGCGGGCAAGACCTTTCTCCTTCACGGGGTGGAAGGTC (SEQ ID NO: 559)
WB	CTAGTTTTAAAGTTGGTTATTAGTTACTGTGATATTTATCACGGTACCCAATAACCAATGAAT (SEQ ID NO: 560)	CGGAAGGTAGCGTCAACGATAGGTGTAACTGTCGTGTTTGTAACGGTACTTCCAACAGCTGGCGCCGCCAC (SEQ ID NO: 561)
BL3	CAATGAAAAACTAGGCATGTAGAAGTTGTTTGT (SEQ ID NO: 562)	TTTCCACAGACAACTCACGTGGAGGTAGTCAC (SEQ ID NO: 563)
SprA	TGTAGTAAGTATCTTAATATACAGCTTTATCTGTTTTTTAAGATACTTACTACTTT (SEQ ID NO: 564)	CACCCATTGTGTTCACAGGAGATACAGCTTTATCTGTACTGATATTAATGACATGCTG (SEQ ID NO: 565)
phiJoe	AGTTGTGGCCATGTGTCCATCTGGGGGCAGATGGAGACGGGGTCACA (SEQ ID NO: 566)	ATCTGGATGTGGGTGTCCATCTGCGGGCAGACGCCGCAGTCGAAGCACGG (SEQ ID NO: 567)
--	--	ACCTTGATCTCGGTGTCCATCGCCGGGCAGACGCCGCAGTCGAAGCACGG (SEQ ID NO: 568)
phiK38	CCCTAATACGCAAGTCGATAACTCTCCTGGGAGCGTTGACAACTTGCGCACCCTGATCTG (SEQ ID NO: 569)	GAGCGCCGGATCAGGGAGTGGACGGCCTGGGAGCGCTACACGCTGTGGCTGCGGTCGGTGC (SEQ ID NO: 570)
Int2	GCTCATGTATGTGTCTACGCGAGATTCTCGCCCGAGAACTTCTGCAAGGCACTGCTCTTGGCT (SEQ ID NO: 571)	GGACGGCGCAGAAGGGGAGTAGCTCTTCGCCGGACCGTCGACATACTGCTCAGCTCGTC (SEQ ID NO: 572)
Int3	ATGGATAAAAAAATACAGCGTTTTTCATGTACAACTATACTAGTTGTAGTGCCTAAATAATGCTT (SEQ ID NO: 573)	GTTTGTAAAGGAGACTGATAATGGCATGTACAACTATACTCGTCGGTAAAAAGGCATCTTAT (SEQ ID NO: 574)
Int4	AAAAATTACAAAGTTTTCAACCCTTGATTTGAATTAGCGGTCAAATAATTTGTAATTCGTTT (SEQ ID NO: 575)	TTCCAAAGAGCGCCCAACGCGACCTGAAATTTGAATAAGACTGCTGCTTGTGTAAAGGCGATGATT (SEQ ID NO: 576)
Int7	GTGTTATAAACCTGTGTGAGAGTTAAGTTTACATGCCTAACCTT	AGACGAGAAACGTTCCGTCCGTCTGGGTCAGTTGGGCAAAGTTGATGA

Recombinase	Recombinase recognition sequence Left	Recombinase recognition sequence Right
	AACTTTTACGCAGGTTCAGCTT (SEQ ID NO: 577)	CCGGGTCGTCCGTT (SEQ ID NO: 578)
Int8	TTAATAAACTATGGAAGTATGT ACAGTCTTGCAATGTTGAGTGA ACAAACTTCCATAATAAAAT (SEQ ID NO: 579)	CAATCATCAGATAACTATGGCGGC ACGTGCATTAACCACGGTTGTATCC CGTCTAAAGTACTCGT (SEQ ID NO: 580)
Int9	GTGGTTGTTTTTGTTGGAAGTG TGTATCAGGTATCTGCATAGTT ATTCCGAACTTCCAATTA (SEQ ID NO: 581)	TTTATATTGCGAAAAATAATTGGC GAACGAGGTAACTGGATACCTCAT CCGCCAATTAAAATTTG (SEQ ID NO: 582)
Int10	GGAAAATATAAATAATTTTAGT AACCTACATCTCAATCAAGGAT AGTAAAACTCTCACTCTT (SEQ ID NO: 583)	AGCACGCTGATAATCAGCAAGACC ACCAACATTTCCACCAATGTAAAA GCTTTAACCTTAGC (SEQ ID NO: 584)
Int11	GTTTATATGTTTACTAATAAGA CGCTCTCAACCCATAAAGTCTT ATTAGTAAACATATTTCAACT (SEQ ID NO: 585)	ATGGATTTTGCAGATTCCCAGATGC CCCTACAGAAAGAGGTACAAAACA TTTATTGGAATTAATT (SEQ ID NO: 586)
Int12	TTTTTGTATGTTAGTTGTGTCA CTGGGTAGACCTAAATAGTGA CACAACTGCTATTAAAATTTAA (SEQ ID NO: 587)	GTTCGTGGTAACTATGGGTGGTAC AGGTGCCACATTAGTTGTACCATTT ATGTTTATGTGGTTAAC (SEQ ID NO: 588)
Int13	CAATAACGGTTGTATTTGTAGA ACTTGACCAGTTGTTTTAGTAA CATAAATACAACTCCGAATA (SEQ ID NO: 589)	GCATACATTGTTGTTGTTTTTCCAG ATCCAGTTGGTCCTGTAAATATAA GCAATCCATGTGAGT (SEQ ID NO: 590)
LI	GTTTAGTATCTCGTTATCTCTC GTTGGAGGGAGAAGAAACGGG ATACCAAAA (SEQ ID NO: 591)	TAACTTTTTCGGATCGAGTTATGAT GGACGTAAAGAGGGAACAAAGCA TCTA (SEQ ID NO: 592)
Peaches	TAGTTTCCAATGTTACAGGAAC TGCTGGCAGAATCCAACACATT GGAAGTCG (SEQ ID NO: 593)	CGGTCTCCATCGGGATCTGCTGATC GAGCAGCATGCCGACCA (SEQ ID NO: 594)
Bxz2	TAACCGCAAGTGTACATCCCTC GGCTGGCCGAGACAAGTACAG TTGCGACAG (SEQ ID NO: 595)	CGGTCTCCATCGGGATCTGCTGATC GAGCAGCATGCCGACCA (SEQ ID NO: 596)
SV1	ATGTGGTCCTTTAGATCCACTG ACGTGGGTCAGTGTCTCTAAAG GACTCGCG (SEQ ID NO: 597)	CATCAGGGCGGTCAGGCCGTAGAT GTGGAAGAAACGGCAGCACGGCG AGGACG (SEQ ID NO: 598)

Table 9. Serine resolvases and SSR target sequences.

Resolvase	Recombinase recognition sequence Left	Recombinase recognition sequence Right
Gin	CGTTTCCTGTAAACCGAGGTTTTG GATAAACA (SEQ ID NO: 599)	CGTTTCCTGTAAACCGAGGTTTTG GATAATGG (SEQ ID NO: 600)
Cin	GAGTTCTCTTAAACCAAGGTTTA GGATTGAAA (SEQ ID NO: 601)	GAGTTCTCTTAAACCAAGGTATT GGATAACAG (SEQ ID NO: 602)
Hin	TGGTTCTTGAAAACCAAGGTTTTT GATAAAGC (SEQ ID NO: 603)	AAATTTTCCTTTTTGGAAGGTTTT TGATAACCA (SEQ ID NO: 604)
Min	GCCTTCCCCTAAACCAACGTTTTT ATGCCGCC (SEQ ID NO: 605)	GCCTTCCCCCAAACCAAGGTAAT CAAGAACGC (SEQ ID NO: 606)
Sin	TTGTGAAATTTGGGTACACCCTA ATCATACAA (SEQ ID NO: 607)	CGTATGATTAGGGTGTATATTAA TTT (SEQ ID NO: 608)

Table 10. Tyrosine integrases and target sequences.

Integrase	*attP*	*attB*
HK022	CAAATGATTTTATTTTGACTAATAA TGACCTACTTACATTAATTTACTGAT AATTAAAGAGATTTTAAATATACAA CTTATTCACCTAAAGGATGACAAAA (SEQ ID NO: 609)	GCACTTTAGGTGAAAAAGGT T (SEQ ID NO: 610)
	TAACATTAATCACTTAAAAATCATC GCATTACACTAATCTGTGGTTAAAT GATAGACTACATAATGCGACAAAA CGCAACATATCCAGTCACTATGAAT CAACTACTTAGATAGTATTAGTGAC CT (SEQ ID NO: 611)	
P22	CTAAGTGGTTTGGGACAAAAATGGG ACATACAAATCTTTGCATCGGTTTG CAAGGCTTTGCATGTCTTTCGAAGA TGGGACGTGTGAGCGCAGGTATGAC GTGGTATGTGTTGACTTAAAAGGTA GTTCTTATAATTCGTAATGCGAAGG TCGTAGGTTCGACTCCTATTATCGG CACCAGTTAAATCAAATACTTACGT ATTATTCGTGCCTTCCTTATTTTTAC TGTGGGACATATTTGGGACAGAAGT ACCAAAAA (SEQ ID NO: 612)	GCAGCGCATTCGTAATGCGA AGGTCGT (SEQ ID NO: 613)
L5	GCGATCCCCATCCGCGACGTGCCAA CTAGGTCTCCTCTCGTCGTGAACAA GGCTACCGGGTTGCAACTCCTGTGC AACTCTCAGGCTTCAACGCGCTTCT ACGACCTGCAATTTCTTTCCACTTA	GAGCGGGCGACGGGAATCG AACCCGCGTAGCTAGTTTGG AAGA (SEQ ID NO: 615)

	GAGGATGCAGCCGAGAGGGGTAAA AACCTATCTTGACCGGCCCATATGT GGTCGGCAGACACCCATTCTTCCAA ACTAGCTACGCGGGTTCGATTCCCG TCGCCCGCTCCGCTGGTCAGAGGGT GTTTTCGCCCTCTGGCCATTTTTCTT TCCAGGGGTCTGCAACTCTTGTGCG ACTCTTCTGACCTGGGCATACGCGG TTGCAACGCATCCCTGATCTGGCTA CTTTCGATGCTGACAAACGAATAGA GCCCCCCGCCTGCGCGAACAGACG AGGGGCATTCACA (SEQ ID NO: 614)	

[1022] In various other aspects, the present disclosure relates to methods of using PE to install one or more recombinase recognition sequence and their use in site-specific recombination.

[1023] In some embodiments, the site-specific recombination may effecuate a variety of recombination outcomes, such as, excision, integration, inversion, or exhange of DNA fragments.

[1024] In some embodiments, the methods are useful for inducing recombination of or between two or more regions of two or more nucleic acid (e.g., DNA) molecules. In other embodiments, the methods are useful for inducing recombination of or between two or more regions in a single nucleic acid molecule (e.g., DNA).

[1025] In some embodiments, the disclosure provides a method for integrating a donor DNA template by site-specific recombination, comprising: (a) installing a recombinase recognition sequence at a genomic locus by prime editing; (b) contacting the genomic locus with a DNA donor template that also comprises the recombinase recognition sequence in the presence of a recombinase.

[1026] In other embodiments, the disclosure provides a method for deleting a genomic region by site-specific recombination, comprising: (a) installing a pair of recombinase recognition sequences at a genomic locus by prime editing; (b) contacting the genomic locus with a recombinase, thereby catalyzing the deletion of the genomic region between the pair of recombinase recognition sequences.

[1027] In yet other embodiments, the disclosure provides a method for inverting a genomic region by site-specific recombination, comprising: (a) installing a pair of recombinase recognition sequences at a genomic locus by prime editing; (b) contacting the genomic locus

with a recombinase, thereby catalyzing the inversion of the genomic region between the pair of recombinase recognition sequences.

[1028] In still other embodiments, the disclosure provides a method for inducing chromosomal translocation between a first genomic site and a second genomic site, comprising: (a) installing a first recombinase recognition sequence at a first genomic locus by prime editing; (b) installing a second recombinase recognition sequence at a second genomic locus by prime editing; (c) contacting the first and the second genomic loci with a recombinase, thereby catalyzing the chromosomal translocation of the first and second genomic loci.

[1029] In other embodiments, the disclosure provides a method for inducing cassette exchange between a genomic locus and a donor DNA comprising a cassette, comprising: (a) installing a first recombinase recognition sequence at a first genomic locus by prime editing; (b) installing a second recombinase recognition sequence at a second genomic locus by prime editing; (c) contacting the first and the second genomic loci with a donor DNA comprising a cassette that is flanked by the first and scond recombinase recognition sequences and a recombinase, thereby catalyzing the exchange of the flanked genomic locus and the cassette in the DNA donor.

[1030] In various embodiments involving the insertion of more than one recombinase recognition sequences in the genome, the recombinase recognition sequences can be the same or different. In some embodiments, the recombinase recognition sequences are the same. In other embodiments, that recombinase recognition sequences are different.

[1031] In various embodiments, the recombinase can be a tyrosine recombinase, such as Cre, Dre, Vcre, Scre, Flp, B2, B3, Kw, R, TD1-40, Vika, Nigri, Panto, Kd, Fre, Cre(ALSHG), Tre, Brec1, or Cre-R3M3, as shown in Table 7. In such embodiments, the recombinase recognition sequence may be an RRS of Table 7 that corresponds to the recombinase under use.

[1032] In various other embodiments, the recombinase can be a large serine recombinase, such as Bxb1, PhiC31, R4, phiBT1, MJ1, MR11, TP901-1, A118, V153, phiRV1, phi370.1, TG1, WB, BL3, SprA, phiJoe, phiK38, Int2, Int3, Int4, Int7, Int8, Int9, Int10, Int11, Int12, Int13, L1, peaches, Bxz2, or SV1, as shown in Table 8. In such embodiments, the recombinase recognition sequence may be an RRS of Table 8 that corresponds to the recombinase under use.

[1033] In still other embodiments, the recombinase can be a serine recombinase, such as Bxb1, PhiC31, R4, phiBT1, MJ1, MR11, TP901-1, A118, V153, phiRV1, phi370.1, TG1, WB, BL3, SprA, phiJoe, phiK38, Int2, Int3, Int4, Int7, Int8, Int9, Int10, Int11, Int12, Int13, L1, peaches, Bxz2, or SV1, as shown in Table 8. In such embodiments, the recombinase recognition sequence may be an RRS of Table 8 that corresponds to the recombinase under use.

[1034] In other embodiments, the recombinase can be a serine resolvase, such as Gin, Cin, Hin, Min, or Sin, as shown in Table 9. In such embodiments, the recombinase recognition sequence may be an RRS of Table 9 that corresponds to the recombinase under use.

[1035] In various other embodiments, the recombinase can be a tyrosine integrase, such as HK022, P22, or L5, as shown in Table 10. In such embodiments, the recombinase recognition sequence may be an RRS of Table 10 that corresponds to the recombinase under use.

[1036] In some embodiments, any of the methods for site-specific recombination with PE can be performed *in vivo* or *in vitro*. In some embodiments, any of the methods for site-specific recombination are performed in a cell (e.g., recombine genomic DNA in a cell). The cell can be prokaryotic or eukaryotic. The cell, such as a eukaryotic cell, can be in an individual, such as a subject, as described herein (e.g., a human subject). The methods described herein are useful for the genetic modification of cells *in vitro* and *in vivo*, for example, in the context of the generation of transgenic cells, cell lines, or animals, or in the alteration of genomic sequence, e.g., the correction of a genetic defect, in a cell in a subject.

References cited for Section L

[1037] Each of the following references are cited in Example 17, each of which are incorporated herein by reference.

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3. Shaw, C.J. & Lupski, J.R. Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. Hum Mol Genet 13 Spec No 1, R57-64 (2004).

4. Carvalho, C.M., Zhang, F. & Lupski, J.R. Evolution in health and medicine Sackler colloquium: Genomic disorders: a window into human gene and genome evolution. Proc Natl Acad Sci U S A 107 Suppl 1, 1765-1771 (2010).

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6. Aplan, P.D. Causes of oncogenic chromosomal translocation. Trends Genet 22, 46-55 (2006).

7. McCarroll, S.A. & Altshuler, D.M. Copy-number variation and association studies of human disease. Nat Genet 39, S37-42 (2007).

[8] Methods of treatment

[1038] The instant disclosure provides methods for the treatment of a subject diagnosed with a disease associated with or caused by a point mutation, or other mutations (e.g., deletion, insertion, inversion, duplication, etc.) that can be corrected by the prime editing system provided herein, as exemplified, but not limited to prion disease (e.g., Example 5 herein), trinucleotide repeat expansion disease (e.g., Example 3 herein), or CDKL5 Deficiency Disorder (CDD) (e.g., Example 23 herein).

[1039] Virtually any disease-causing genetic defect may be repaired by using prime editing, which includes the selection of an appropriate prime editor fusion protein (including a napDNAbp and a polymerase (e.g., a reverse transcriptase), and designing of an appropriate PEgRNA designed to (a) target the appropriate target DNA containing an edit site, and (b) provide a template for the synthesis of a single strand of DNA from the 3' end of the nick site that includes the desired edit which displaces and replaces the endogenous strand immediately downstream of the nick site. Prime editing can be used, without limitation, to (a) install mutation-correcting changes to a nucleotide sequence, (b) install protein and RNA tags, (c) install immunoepitopes on proteins of interest, (d) install inducible dimerization domains in proteins, (e) install or remove sequences to alter that activity of a biomolecule, (f) install recombinase target sites to direct specific genetic changes, and (g) mutagenesis of a target sequence by using an error-prone RT.

[1040] The method of treating a disorder can involve as an early step the design of an appropriate PEgRNA and prime editor fusion protein in accordance with the methods described herein, which include a number of considerations that may be taken into account, such as:

(a) the target sequence, i.e., the nucleotide sequence in which one or more nucleobase modifications are desired to be installed by the prime editor;
(b) the location of the cut site within the target sequence, i.e., the specific nucleobase position at which the prime editor will induce a single-stand nick to create a 3′ end RT primer sequence on one side of the nick and the 5′ end endogenous flap on the other side of the nick (which ultimately is removed by FEN1 or equivalent thereto and replaced by the 3′ ssDNA flap. The cut site creates the 3′ end primer sequence which becomes extended by the polymerase of the PE fusion protein (e.g., a RT enzyme) during RNA-dependent DNA polymerization to create the 3′ ssDNA flap containing the desired edit, which then replaces the 5′ endogenous DNA flap in the target sequence.
(c) the available PAM sequences (including the canonical SpCas9 PAM sites, as well as non-canonical PAM sites recognized by Cas9 variants and equivalents with expanded or differing PAM specificities);
(d) the spacing between the available PAM sequences and the location of the cut site in the PAM strand;
(e) the particular Cas9, Cas9 variant, or Cas9 equivalent of the prime editor available to be used (which in part is dictated by the available PAM);
(f) the sequence and length of the primer binding site;
(g) the sequence and length of the edit template;
(h) the sequence and length of the homology arm;
(i) the spacer sequence and length; and
(j) the gRNA core sequence.

[1041] A suitable PEgRNA, and optionally a nicking-sgRNA design guide for second-site nicking, can be designed by way of the following exemplarly step-by-step set of instructions which takes into account one or more of the above considerations. The steps reference the examples shown in FIGs. 70A-70I.

1. **Define the target sequence and the edit**. Retrieve the sequence of the target DNA region (~200bp) centered around the location of the desired edit (point mutation, insertion, deletion, or combination thereof). See FIG. 70A.

2. **Locate target PAMs**. Identify PAMs in the proximity to the desired edit location. PAMs can be identified on either strand of DNA proximal to the desired edit location. While PAMs close to the edit position are preferred (i.e., wherein the nick site is less than 30 nt from the edit position, or less than 29 nt, 28 nt, 27 nt, 26 nt, 25 nt, 24 nt, 23 nt, 22 nt, 21 nt,

20 nt, 19 nt, 18 nt, 17 nt, 16 nt, 15 nt, 14 nt, 13 nt, 12 nt, 11 nt, 10 nt, 9 nt, 8 nt, 7 nt, 6 nt, 5 nt, 4 nt, 3 nt, or 2 nt from the edit position to the nick site), it is possible to install edits using protospacers and PAMs that place the nick ≥ 30 nt from the edit position. See FIG. 70B.

3. **Locate the nick sites**. For each PAM being considered, identify the corresponding nick site and on which strand. For Sp Cas9 H840A nickase, cleavage occurs in the PAM-containing strand between the 3rd and 4th bases 5′ to the NGG PAM. All edited nucleotides must exist 3′ of the nick site, so appropriate PAMs must place the nick 5′ to the target edit on the PAM-containing strand. In the example shown below, there are two possible PAMs. For simplicity, the remaining steps will demonstrate the design of a PEgRNA using PAM 1 only. See FIG. 70C.

4. **Design the spacer sequence**. The protospacer of SpCas9 corresponds to the 20 nucleotides 5′ to the NGG PAM on the PAM-containing strand. Efficient Pol III transcription initiation requires a G to be the first transcribed nucleotide. If the first nucleotide of the protospacer is a G, the spacer sequence for the PEgRNA is simply the protospacer sequence. If the first nucleotide of the protospacer is not a G, the spacer sequence of the PEgRNA is G followed by the protospacer sequence. See FIG. 70D.

5. **Design a primer binding site (PBS)**. Using the starting allele sequence, identify the DNA primer on the PAM-containing strand. The 3′ end of the DNA primer is the nucleotide just upstream of the nick site (i.e. the 4th base 5′ to the NGG PAM for Sp Cas9). As a general design principle for use with PE2 and PE3, a PEgRNA primer binding site (PBS) containing 12 to 13 nucleotides of complementarity to the DNA primer can be used for sequences that contain ~40-60% GC content. For sequences with low GC content, longer (14- to 15-nt) PBSs should be tested. For sequences with higher GC content, shorter (8- to 11-nt) PBSs should be tested. Optimal PBS sequences should be determined empirically, regardless of GC content. To design a length-*p* PBS sequence, take the reverse complement of the first *p* nucleotides 5′ of the nick site in the PAM-containing strand using the *starting* allele sequence. See FIG. 70E.

6. **Design an RT template (or DNA synthesis template)**. The RT template (or DNA synthesis template where the polymerase is not reverse transcriptase) encodes the designed edit and homology to the sequence adjacent to the edit. In one embodiment, these regions correspond to the DNA synthesis template of FIG. 3D and FIG. 3E, wherein the DNA synthesis template comprises the "edit template" and the "homology arm." Optimal RT template lengths vary based on the target site. For short-range edits (positions +1 to +6), it is

recommended to test a short (9 to 12 nt), a medium (13 to 16 nt), and a long (17 to 20 nt) RT template. For long-range edits (positions +7 and beyond), it is recommended to use RT templates that extend at least 5 nt (preferably 10 or more nt) past the position of the edit to allow for sufficient 3′ DNA flap homology. For long-range edits, several RT templates should be screened to identify functional designs. For larger insertions and deletions (≥5 nt), incorporation of greater 3′ homology (~20 nt or more) into the RT template is recommended. Editing efficiency is typically impaired when the RT template encodes the synthesis of a G as the last nucleotide in the reverse transcribed DNA product (corresponding to a C in the RT template of the PEgRNA). As many RT templates support efficient prime editing, avoidance of G as the final synthesized nucleotide is recommended when designing RT templates. To design a length-*r* RT template sequence, use the *desired* allele sequence and take the reverse complement of the first *r* nucleotides 3′ of the nick site in the strand that originally contained the PAM. Note that compared to SNP edits, insertion or deletion edits using RT templates of the same length will not contain identical homology. See FIG. 70F.

7. **Assemble the full PEgRNA sequence**. Concatenate the PEgRNA components in the following order (5′ to 3′): spacer, scaffold, RT template and PBS. See FIG. 70G.

8. **Designing nicking-sgRNAs for PE3**. Identify PAMs on the non-edited strand upstream and downstream of the edit. Optimal nicking positions are highly locus-dependent and should be determined empirically. In general, nicks placed 40 to 90 nucleotides 5′ to the position across from the PEgRNA-induced nick lead to higher editing yields and fewer indels. A nicking sgRNA has a spacer sequence that matches the 20-nt protospacer in the *starting* allele, with the addition of a 5′-G if the protospacer does not begin with a G. See FIG. 70H.

9. **Designing PE3b nicking-sgRNAs**. If a PAM exists in the complementary strand and its corresponding protospacer overlaps with the sequence targeted for editing, this edit could be a candidate for the PE3b system. In the PE3b system, the spacer sequence of the nicking-sgRNA matches the sequence of the desired edited allele, but not the starting allele. The PE3b system operates efficiently when the edited nucleotide(s) falls within the seed region (~10 nt adjacent to the PAM) of the nicking-sgRNA protospacer. This prevents nicking of the complementary strand until after installation of the edited strand, preventing competition between the PEgRNA and the sgRNA for binding the target DNA. PE3b also avoids the generation of simultaneous nicks on both strands, thus reducing indel formation significantly while maintaining high editing efficiency. PE3b sgRNAs should have a spacer sequence that

matches the 20-nt protospacer in the *desired* allele, with the addition of a 5′ G if needed. See FIG. 70I.

[1042] The above step-by-step process for designing a suitable PEgRNA and a second-site nicking sgRNA is not meant to be limiting in any way. The disclosure contemplates variations of the above-described step-by-step process which would be derivable therefrom by a person of ordinary skill in the art.

[1043] Once a suitable PEgRNA and PE fusion protein are selected/designed, they may be administered by a suitable methodology, such as by vector-based transfection (in which one or more vectors comprising DNA encoding the PEgRNA and the PE fusion protein and which are expressed within a cell upon trransfection with the vectors), direct delivery of the PE fusion protein complexed with the PEgRNA (e.g., RNP delivery) in a delivery format (e.g., lipid particles, nanoparticles), or by a mRNA-based delivery system. Such methods are described herein in the present disclosure and any know method may be utilized.

[1044] The PEgRNA and PE fusion protein (or together, referred to as the PE complex) can be delivered to a cell in a therapeutically effective amount such that upon contacting the target DNA of interest, the desired edit becomes installed therein.

[1045] Any disease is conceivably treatable by such methods so long as delivery to the appropriate cells is feasible. The person having ordinary skill in the art will be able to choose and/or select a PE delivery methodology to suit the intended purpose and the intended target cells.

[1046] For example, in some embodiments, a method is provided that comprises administering to a subject having such a disease, e.g., a cancer associated with a point mutation as described above, an effective amount of the prime editing system described herein that corrects the point mutation or introduces a deactivating mutation into a disease-associated gene as mediated by homology-directed repair in the presence of a donor DNA molecule comprising desired genetic change. In some embodiments, a method is provided that comprises administering to a subject having such a disease, e.g., a cancer associated with a point mutation as described above, an effective amount of the prime editing system described herein that corrects the point mutation or introduces a deactivating mutation into a disease-associated gene. In some embodiments, the disease is a proliferative disease. In some embodiments, the disease is a genetic disease. In some embodiments, the disease is a neoplastic disease. In some embodiments, the disease is a metabolic disease. In some embodiments, the disease is a lysosomal storage disease. Other diseases that can be treated

by correcting a point mutation or introducing a deactivating mutation into a disease-associated gene will be known to those of skill in the art, and the disclosure is not limited in this respect.

[1047] The instant disclosure provides methods for the treatment of additional diseases or disorders, e.g., diseases or disorders that are associated or caused by a point mutation that can be corrected by TPRT-mediated gene editing. Some such diseases are described herein, and additional suitable diseases that can be treated with the strategies and fusion proteins provided herein will be apparent to those of skill in the art based on the instant disclosure. Exemplary suitable diseases and disorders are listed below. It will be understood that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used. Numbering might be different, e.g., in precursors of a mature protein and the mature protein itself, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues. Exemplary suitable diseases and disorders include, without limitation: 2-methyl-3-hydroxybutyric aciduria; 3 beta-Hydroxysteroid dehydrogenase deficiency; 3-Methylglutaconic aciduria; 3-Oxo-5 alpha-steroid delta 4-dehydrogenase deficiency; 46,XY sex reversal, type 1, 3, and 5; 5-Oxoprolinase deficiency; 6-pyruvoyl-tetrahydropterin synthase deficiency; Aarskog syndrome; Aase syndrome; Achondrogenesis type 2; Achromatopsia 2 and 7; Acquired long QT syndrome; Acrocallosal syndrome, Schinzel type; Acrocapitofemoral dysplasia; Acrodysostosis 2, with or without hormone resistance; Acroerythrokeratoderma; Acromicric dysplasia; Acth-independent macronodular adrenal hyperplasia 2; Activated PI3K-delta syndrome; Acute intermittent porphyria; deficiency of Acyl-CoA dehydrogenase family, member 9; Adams-Oliver syndrome 5 and 6; Adenine phosphoribosyltransferase deficiency; Adenylate kinase deficiency; hemolytic anemia due to Adenylosuccinate lyase deficiency; Adolescent nephronophthisis; Renal-hepatic-pancreatic dysplasia; Meckel syndrome type 7; Adrenoleukodystrophy; Adult junctional epidermolysis bullosa; Epidermolysis bullosa, junctional, localisata variant; Adult neuronal ceroid lipofuscinosis; Adult neuronal ceroid lipofuscinosis; Adult onset ataxia with oculomotor apraxia; ADULT syndrome; Afibrinogenemia and congenital Afibrinogenemia; autosomal recessive Agammaglobulinemia 2; Age-related macular degeneration 3, 6, 11, and 12; Aicardi Goutieres syndromes 1, 4, and 5; Chilbain lupus 1; Alagille syndromes 1 and 2;

Alexander disease; Alkaptonuria; Allan-Herndon-Dudley syndrome; Alopecia universalis congenital; Alpers encephalopathy; Alpha-1-antitrypsin deficiency; autosomal dominant, autosomal recessive, and X-linked recessive Alport syndromes; Alzheimer disease, familial, 3, with spastic paraparesis and apraxia; Alzheimer disease, types, 1, 3, and 4; hypocalcification type and hypomaturation type, IIA1 Amelogenesis imperfecta; Aminoacylase 1 deficiency; Amish infantile epilepsy syndrome; Amyloidogenic transthyretin amyloidosis; Amyloid Cardiomyopathy, Transthyretin-related; Cardiomyopathy; Amyotrophic lateral sclerosis types 1, 6, 15 (with or without frontotemporal dementia), 22 (with or without frontotemporal dementia), and 10; Frontotemporal dementia with TDP43 inclusions, TARDBP-related; Andermann syndrome; Andersen Tawil syndrome; Congenital long QT syndrome; Anemia, nonspherocytic hemolytic, due to G6PD deficiency; Angelman syndrome; Severe neonatal-onset encephalopathy with microcephaly; susceptibility to Autism, X-linked 3; Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps; Angiotensin i-converting enzyme, benign serum increase; Aniridia, cerebellar ataxia, and mental retardation; Anonychia; Antithrombin III deficiency; Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis; Aortic aneurysm, familial thoracic 4, 6, and 9; Thoracic aortic aneurysms and aortic dissections; Multisystemic smooth muscle dysfunction syndrome; Moyamoya disease 5; Aplastic anemia; Apparent mineralocorticoid excess; Arginase deficiency; Argininosuccinate lyase deficiency; Aromatase deficiency; Arrhythmogenic right ventricular cardiomyopathy types 5, 8, and 10; Primary familial hypertrophic cardiomyopathy; Arthrogryposis multiplex congenita, distal, X-linked; Arthrogryposis renal dysfunction cholestasis syndrome; Arthrogryposis, renal dysfunction, and cholestasis 2; Asparagine synthetase deficiency; Abnormality of neuronal migration; Ataxia with vitamin E deficiency; Ataxia, sensory, autosomal dominant; Ataxia-telangiectasia syndrome; Hereditary cancer-predisposing syndrome; Atransferrinemia; Atrial fibrillation, familial, 11, 12, 13, and 16; Atrial septal defects 2, 4, and 7 (with or without atrioventricular conduction defects); Atrial standstill 2; Atrioventricular septal defect 4; Atrophia bulborum hereditaria; ATR-X syndrome; Auriculocondylar syndrome 2; Autoimmune disease, multisystem, infantile-onset; Autoimmune lymphoproliferative syndrome, type 1a; Autosomal dominant hypohidrotic ectodermal dysplasia; Autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions 1 and 3; Autosomal dominant torsion dystonia 4; Autosomal recessive centronuclear myopathy; Autosomal recessive congenital ichthyosis 1, 2, 3, 4A, and 4B; Autosomal recessive cutis

laxa type IA and 1B; Autosomal recessive hypohidrotic ectodermal dysplasia syndrome; Ectodermal dysplasia 11b; hypohidrotic/hair/tooth type, autosomal recessive; Autosomal recessive hypophosphatemic bone disease; Axenfeld-Rieger syndrome type 3; Bainbridge-Ropers syndrome; Bannayan-Riley-Ruvalcaba syndrome; PTEN hamartoma tumor syndrome; Baraitser-Winter syndromes 1 and 2; Barakat syndrome; Bardet-Biedl syndromes 1, 11, 16, and 19; Bare lymphocyte syndrome type 2, complementation group E; Bartter syndrome antenatal type 2; Bartter syndrome types 3, 3 with hypocalciuria , and 4; Basal ganglia calcification, idiopathic, 4; Beaded hair; Benign familial hematuria; Benign familial neonatal seizures 1 and 2; Seizures, benign familial neonatal, 1, and/or myokymia; Seizures, Early infantile epileptic encephalopathy 7; Benign familial neonatal-infantile seizures; Benign hereditary chorea; Benign scapuloperoneal muscular dystrophy with cardiomyopathy; Bernard-Soulier syndrome, types A1 and A2 (autosomal dominant); Bestrophinopathy, autosomal recessive; beta Thalassemia; Bethlem myopathy and Bethlem myopathy 2; Bietti crystalline corneoretinal dystrophy; Bile acid synthesis defect, congenital, 2; Biotinidase deficiency; Birk Barel mental retardation dysmorphism syndrome; Blepharophimosis, ptosis, and epicanthus inversus; Bloom syndrome; Borjeson-Forssman-Lehmann syndrome; Boucher Neuhauser syndrome; Brachydactyly types A1 and A2; Brachydactyly with hypertension; Brain small vessel disease with hemorrhage; Branched-chain ketoacid dehydrogenase kinase deficiency; Branchiootic syndromes 2 and 3; Breast cancer, early-onset; Breast-ovarian cancer, familial 1, 2, and 4; Brittle cornea syndrome 2; Brody myopathy; Bronchiectasis with or without elevated sweat chloride 3; Brown-Vialetto-Van laere syndrome and Brown-Vialetto-Van Laere syndrome 2; Brugada syndrome; Brugada syndrome 1; Ventricular fibrillation; Paroxysmal familial ventricular fibrillation; Brugada syndrome and Brugada syndrome 4; Long QT syndrome; Sudden cardiac death; Bull eye macular dystrophy; Stargardt disease 4; Cone-rod dystrophy 12; Bullous ichthyosiform erythroderma; Burn-Mckeown syndrome; Candidiasis, familial, 2, 5, 6, and 8; Carbohydrate-deficient glycoprotein syndrome type I and II; Carbonic anhydrase VA deficiency, hyperammonemia due to; Carcinoma of colon; Cardiac arrhythmia; Long QT syndrome, LQT1 subtype; Cardioencephalomyopathy, fatal infantile, due to cytochrome c oxidase deficiency; Cardiofaciocutaneous syndrome; Cardiomyopathy; Danon disease; Hypertrophic cardiomyopathy; Left ventricular noncompaction cardiomyopathy; Carnevale syndrome; Carney complex, type 1; Carnitine acylcarnitine translocase deficiency; Carnitine palmitoyltransferase I , II, II (late onset), and II (infantile) deficiency; Cataract 1, 4,

autosomal dominant, autosomal dominant, multiple types, with microcornea, coppock-like, juvenile, with microcornea and glucosuria, and nuclear diffuse nonprogressive; Catecholaminergic polymorphic ventricular tachycardia; Caudal regression syndrome; Cd8 deficiency, familial; Central core disease; Centromeric instability of chromosomes 1,9 and 16 and immunodeficiency; Cerebellar ataxia infantile with progressive external ophthalmoplegi and Cerebellar ataxia, mental retardation, and dysequilibrium syndrome 2; Cerebral amyloid angiopathy, APP-related; Cerebral autosomal dominant and recessive arteriopathy with subcortical infarcts and leukoencephalopathy; Cerebral cavernous malformations 2; Cerebrooculofacioskeletal syndrome 2; Cerebro-oculo-facio-skeletal syndrome; Cerebroretinal microangiopathy with calcifications and cysts; Ceroid lipofuscinosis neuronal 2, 6, 7, and 10; Ch\xc3\xa9diak-Higashi syndrome , Chediak-Higashi syndrome, adult type; Charcot-Marie-Tooth disease types 1B, 2B2, 2C, 2F, 2I, 2U (axonal), 1C (demyelinating), dominant intermediate C, recessive intermediate A, 2A2, 4C, 4D, 4H, IF, IVF, and X; Scapuloperoneal spinal muscular atrophy; Distal spinal muscular atrophy, congenital nonprogressive; Spinal muscular atrophy, distal, autosomal recessive, 5; CHARGE association; Childhood hypophosphatasia; Adult hypophosphatasia; Cholecystitis; Progressive familial intrahepatic cholestasis 3; Cholestasis, intrahepatic, of pregnancy 3; Cholestanol storage disease; Cholesterol monooxygenase (side-chain cleaving) deficiency; Chondrodysplasia Blomstrand type; Chondrodysplasia punctata 1, X-linked recessive and 2 X-linked dominant; CHOPS syndrome; Chronic granulomatous disease, autosomal recessive cytochrome b-positive, types 1 and 2; Chudley-McCullough syndrome; Ciliary dyskinesia, primary, 7, 11, 15, 20 and 22; Citrullinemia type I; Citrullinemia type I and II; Cleidocranial dysostosis; C-like syndrome; Cockayne syndrome type A, ; Coenzyme Q10 deficiency, primary 1, 4, and 7; Coffin Siris/Intellectual Disability; Coffin-Lowry syndrome; Cohen syndrome, ; Cold-induced sweating syndrome 1; COLE-CARPENTER SYNDROME 2; Combined cellular and humoral immune defects with granulomas; Combined d-2- and l-2-hydroxyglutaric aciduria; Combined malonic and methylmalonic aciduria; Combined oxidative phosphorylation deficiencies 1, 3, 4, 12, 15, and 25; Combined partial and complete 17-alpha-hydroxylase/17,20-lyase deficiency; Common variable immunodeficiency 9; Complement component 4, partial deficiency of, due to dysfunctional c1 inhibitor; Complement factor B deficiency; Cone monochromatism; Cone-rod dystrophy 2 and 6; Cone-rod dystrophy amelogenesis imperfecta; Congenital adrenal hyperplasia and Congenital adrenal hypoplasia, X-linked; Congenital amegakaryocytic thrombocytopenia; Congenital

aniridia; Congenital central hypoventilation; Hirschsprung disease 3; Congenital contractural arachnodactyly; Congenital contractures of the limbs and face, hypotonia, and developmental delay; Congenital disorder of glycosylation types 1B, 1D, 1G, 1H, 1J, 1K, 1N, 1P, 2C, 2J, 2K, IIm; Congenital dyserythropoietic anemia, type I and II; Congenital ectodermal dysplasia of face; Congenital erythropoietic porphyria; Congenital generalized lipodystrophy type 2; Congenital heart disease, multiple types, 2; Congenital heart disease; Interrupted aortic arch; Congenital lipomatous overgrowth, vascular malformations, and epidermal nevi; Non-small cell lung cancer; Neoplasm of ovary; Cardiac conduction defect, nonspecific; Congenital microvillous atrophy; Congenital muscular dystrophy; Congenital muscular dystrophy due to partial LAMA2 deficiency; Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies, types A2, A7, A8, A11, and A14; Congenital muscular dystrophy-dystroglycanopathy with mental retardation, types B2, B3, B5, and B15; Congenital muscular dystrophy-dystroglycanopathy without mental retardation, type B5; Congenital muscular hypertrophy-cerebral syndrome; Congenital myasthenic syndrome, acetazolamide-responsive; Congenital myopathy with fiber type disproportion; Congenital ocular coloboma; Congenital stationary night blindness, type 1A, 1B, 1C, 1E, 1F, and 2A; Coproporphyria; Cornea plana 2; Corneal dystrophy, Fuchs endothelial, 4; Corneal endothelial dystrophy type 2; Corneal fragility keratoglobus, blue sclerae and joint hypermobility; Cornelia de Lange syndromes 1 and 5; Coronary artery disease, autosomal dominant 2; Coronary heart disease; Hyperalphalipoproteinemia 2; Cortical dysplasia, complex, with other brain malformations 5 and 6; Cortical malformations, occipital; Corticosteroid-binding globulin deficiency; Corticosterone methyloxidase type 2 deficiency; Costello syndrome; Cowden syndrome 1; Coxa plana; Craniodiaphyseal dysplasia, autosomal dominant; Craniosynostosis 1 and 4; Craniosynostosis and dental anomalies; Creatine deficiency, X-linked; Crouzon syndrome; Cryptophthalmos syndrome; Cryptorchidism, unilateral or bilateral; Cushing symphalangism; Cutaneous malignant melanoma 1; Cutis laxa with osteodystrophy and with severe pulmonary, gastrointestinal, and urinary abnormalities; Cyanosis, transient neonatal and atypical nephropathic; Cystic fibrosis; Cystinuria; Cytochrome c oxidase i deficiency; Cytochrome-c oxidase deficiency ; D-2-hydroxyglutaric aciduria 2; Darier disease, segmental; Deafness with labyrinthine aplasia microtia and microdontia (LAMM); Deafness, autosomal dominant 3a, 4, 12, 13, 15, autosomal dominant nonsyndromic sensorineural 17, 20, and 65; Deafness, autosomal recessive 1A, 2, 3, 6, 8, 9, 12, 15, 16, 18b, 22, 28, 31, 44, 49, 63, 77, 86, and 89; Deafness, cochlear, with myopia and intellectual impairment, without

vestibular involvement, autosomal dominant, X-linked 2; Deficiency of 2-methylbutyryl-CoA dehydrogenase; Deficiency of 3-hydroxyacyl-CoA dehydrogenase; Deficiency of alpha-mannosidase; Deficiency of aromatic-L-amino-acid decarboxylase; Deficiency of bisphosphoglycerate mutase; Deficiency of butyryl-CoA dehydrogenase; Deficiency of ferroxidase; Deficiency of galactokinase; Deficiency of guanidinoacetate methyltransferase; Deficiency of hyaluronoglucosaminidase; Deficiency of ribose-5-phosphate isomerase; Deficiency of steroid 11-beta-monooxygenase; Deficiency of UDPglucose-hexose-1-phosphate uridylyltransferase; Deficiency of xanthine oxidase; Dejerine-Sottas disease; Charcot-Marie-Tooth disease, types ID and IVF; Dejerine-Sottas syndrome, autosomal dominant; Dendritic cell, monocyte, B lymphocyte, and natural killer lymphocyte deficiency; Desbuquois dysplasia 2; Desbuquois syndrome; DFNA 2 Nonsyndromic Hearing Loss; Diabetes mellitus and insipidus with optic atrophy and deafness; Diabetes mellitus, type 2, and insulin-dependent, 20; Diamond-Blackfan anemia 1, 5, 8, and 10; Diarrhea 3 (secretory sodium, congenital, syndromic) and 5 (with tufting enteropathy, congenital); Dicarboxylic aminoaciduria; Diffuse palmoplantar keratoderma, Bothnian type; Digitorenocerebral syndrome; Dihydropteridine reductase deficiency; Dilated cardiomyopathy 1A, 1AA, 1C, 1G, 1BB, 1DD, 1FF, 1HH, 1I, 1KK, 1N, 1S, 1Y, and 3B; Left ventricular noncompaction 3; Disordered steroidogenesis due to cytochrome p450 oxidoreductase deficiency; Distal arthrogryposis type 2B; Distal hereditary motor neuronopathy type 2B; Distal myopathy Markesbery-Griggs type; Distal spinal muscular atrophy, X-linked 3; Distichiasis-lymphedema syndrome; Dominant dystrophic epidermolysis bullosa with absence of skin; Dominant hereditary optic atrophy; Donnai Barrow syndrome; Dopamine beta hydroxylase deficiency; Dopamine receptor d2, reduced brain density of; Dowling-degos disease 4; Doyne honeycomb retinal dystrophy; Malattia leventinese; Duane syndrome type 2; Dubin-Johnson syndrome; Duchenne muscular dystrophy; Becker muscular dystrophy; Dysfibrinogenemia; Dyskeratosis congenita autosomal dominant and autosomal dominant, 3; Dyskeratosis congenita, autosomal recessive, 1, 3, 4, and 5; Dyskeratosis congenita X-linked; Dyskinesia, familial, with facial myokymia; Dysplasminogenemia; Dystonia 2 (torsion, autosomal recessive), 3 (torsion, X-linked), 5 (Dopa-responsive type), 10, 12, 16, 25, 26 (Myoclonic); Seizures, benign familial infantile, 2; Early infantile epileptic encephalopathy 2, 4, 7, 9, 10, 11, 13, and 14; Atypical Rett syndrome; Early T cell progenitor acute lymphoblastic leukemia; Ectodermal dysplasia skin fragility syndrome; Ectodermal dysplasia-syndactyly syndrome 1; Ectopia lentis, isolated autosomal recessive and dominant; Ectrodactyly,

ectodermal dysplasia, and cleft lip/palate syndrome 3; Ehlers-Danlos syndrome type 7 (autosomal recessive), classic type, type 2 (progeroid), hydroxylysine-deficient, type 4, type 4 variant, and due to tenascin-X deficiency; Eichsfeld type congenital muscular dystrophy; Endocrine-cerebroosteodysplasia; Enhanced s-cone syndrome; Enlarged vestibular aqueduct syndrome; Enterokinase deficiency; Epidermodysplasia verruciformis; Epidermolysa bullosa simplex and limb girdle muscular dystrophy, simplex with mottled pigmentation, simplex with pyloric atresia, simplex, autosomal recessive, and with pyloric atresia; Epidermolytic palmoplantar keratoderma; Familial febrile seizures 8; Epilepsy, childhood absence 2, 12 (idiopathic generalized, susceptibility to) 5 (nocturnal frontal lobe), nocturnal frontal lobe type 1, partial, with variable foci, progressive myoclonic 3, and X-linked, with variable learning disabilities and behavior disorders; Epileptic encephalopathy, childhood-onset, early infantile, 1, 19, 23, 25, 30, and 32; Epiphyseal dysplasia, multiple, with myopia and conductive deafness; Episodic ataxia type 2; Episodic pain syndrome, familial, 3; Epstein syndrome; Fechtner syndrome; Erythropoietic protoporphyria; Estrogen resistance; Exudative vitreoretinopathy 6; Fabry disease and Fabry disease, cardiac variant; Factor H, VII, X, v and factor viii, combined deficiency of 2, xiii, a subunit, deficiency; Familial adenomatous polyposis 1 and 3; Familial amyloid nephropathy with urticaria and deafness; Familial cold urticarial; Familial aplasia of the vermis; Familial benign pemphigus; Familial cancer of breast; Breast cancer, susceptibility to; Osteosarcoma; Pancreatic cancer 3; Familial cardiomyopathy; Familial cold autoinflammatory syndrome 2; Familial colorectal cancer; Familial exudative vitreoretinopathy, X-linked; Familial hemiplegic migraine types 1 and 2; Familial hypercholesterolemia; Familial hypertrophic cardiomyopathy 1, 2, 3, 4, 7, 10, 23 and 24; Familial hypokalemia-hypomagnesemia; Familial hypoplastic, glomerulocystic kidney; Familial infantile myasthenia; Familial juvenile gout; Familial Mediterranean fever and Familial mediterranean fever, autosomal dominant; Familial porencephaly; Familial porphyria cutanea tarda; Familial pulmonary capillary hemangiomatosis; Familial renal glucosuria; Familial renal hypouricemia; Familial restrictive cardiomyopathy 1; Familial type 1 and 3 hyperlipoproteinemia; Fanconi anemia, complementation group E, I, N, and O; Fanconi-Bickel syndrome; Favism, susceptibility to; Febrile seizures, familial, 11; Feingold syndrome 1; Fetal hemoglobin quantitative trait locus 1; FG syndrome and FG syndrome 4; Fibrosis of extraocular muscles, congenital, 1, 2, 3a (with or without extraocular involvement), 3b; Fish-eye disease; Fleck corneal dystrophy; Floating-Harbor syndrome; Focal epilepsy with speech disorder with or without mental retardation; Focal segmental

glomerulosclerosis 5; Forebrain defects; Frank Ter Haar syndrome; Borrone Di Rocco Crovato syndrome; Frasier syndrome; Wilms tumor 1; Freeman-Sheldon syndrome; Frontometaphyseal dysplasia 1and 3; Frontotemporal dementia; Frontotemporal dementia and/or amyotrophic lateral sclerosis 3 and 4; Frontotemporal Dementia Chromosome 3-Linked and Frontotemporal dementia ubiquitin-positive; Fructose-biphosphatase deficiency; Fuhrmann syndrome; Gamma-aminobutyric acid transaminase deficiency; Gamstorp-Wohlfart syndrome; Gaucher disease type 1 and Subacute neuronopathic; Gaze palsy, familial horizontal, with progressive scoliosis; Generalized dominant dystrophic epidermolysis bullosa; Generalized epilepsy with febrile seizures plus 3, type 1, type 2; Epileptic encephalopathy Lennox-Gastaut type; Giant axonal neuropathy; Glanzmann thrombasthenia; Glaucoma 1, open angle, e, F, and G; Glaucoma 3, primary congenital, d; Glaucoma, congenital and Glaucoma, congenital, Coloboma; Glaucoma, primary open angle, juvenile-onset; Glioma susceptibility 1; Glucose transporter type 1 deficiency syndrome; Glucose-6-phosphate transport defect; GLUT1 deficiency syndrome 2; Epilepsy, idiopathic generalized, susceptibility to, 12; Glutamate formiminotransferase deficiency; Glutaric acidemia IIA and IIB; Glutaric aciduria, type 1; Gluthathione synthetase deficiency; Glycogen storage disease 0 (muscle), II (adult form), IXa2, IXc, type 1A; type II, type IV, IV (combined hepatic and myopathic), type V, and type VI; Goldmann-Favre syndrome; Gordon syndrome; Gorlin syndrome; Holoprosencephaly sequence; Holoprosencephaly 7; Granulomatous disease, chronic, X-linked, variant; Granulosa cell tumor of the ovary; Gray platelet syndrome; Griscelli syndrome type 3; Groenouw corneal dystrophy type I; Growth and mental retardation, mandibulofacial dysostosis, microcephaly, and cleft palate; Growth hormone deficiency with pituitary anomalies; Growth hormone insensitivity with immunodeficiency; GTP cyclohydrolase I deficiency; Hajdu-Cheney syndrome; Hand foot uterus syndrome; Hearing impairment; Hemangioma, capillary infantile; Hematologic neoplasm; Hemochromatosis type 1, 2B, and 3; Microvascular complications of diabetes 7; Transferrin serum level quantitative trait locus 2; Hemoglobin H disease, nondeletional; Hemolytic anemia, nonspherocytic, due to glucose phosphate isomerase deficiency; Hemophagocytic lymphohistiocytosis, familial, 2; Hemophagocytic lymphohistiocytosis, familial, 3; Heparin cofactor II deficiency; Hereditary acrodermatitis enteropathica; Hereditary breast and ovarian cancer syndrome; Ataxia-telangiectasia-like disorder; Hereditary diffuse gastric cancer; Hereditary diffuse leukoencephalopathy with spheroids; Hereditary factors II, IX, VIII deficiency disease; Hereditary hemorrhagic telangiectasia type

2; Hereditary insensitivity to pain with anhidrosis; Hereditary lymphedema type I; Hereditary motor and sensory neuropathy with optic atrophy; Hereditary myopathy with early respiratory failure; Hereditary neuralgic amyotrophy; Hereditary Nonpolyposis Colorectal Neoplasms; Lynch syndrome I and II; Hereditary pancreatitis; Pancreatitis, chronic, susceptibility to; Hereditary sensory and autonomic neuropathy type IIB amd IIA; Hereditary sideroblastic anemia; Hermansky-Pudlak syndrome 1, 3, 4, and 6; Heterotaxy, visceral, 2, 4, and 6, autosomal; Heterotaxy, visceral, X-linked; Heterotopia; Histiocytic medullary reticulosis; Histiocytosis-lymphadenopathy plus syndrome; Holocarboxylase synthetase deficiency; Holoprosencephaly 2, 3,7, and 9; Holt-Oram syndrome; Homocysteinemia due to MTHFR deficiency, CBS deficiency, and Homocystinuria, pyridoxine-responsive; Homocystinuria-Megaloblastic anemia due to defect in cobalamin metabolism, cblE complementation type; Howel-Evans syndrome; Hurler syndrome; Hutchinson-Gilford syndrome; Hydrocephalus; Hyperammonemia, type III; Hypercholesterolaemia and Hypercholesterolemia, autosomal recessive; Hyperekplexia 2 and Hyperekplexia hereditary; Hyperferritinemia cataract syndrome; Hyperglycinuria; Hyperimmunoglobulin D with periodic fever; Mevalonic aciduria; Hyperimmunoglobulin E syndrome; Hyperinsulinemic hypoglycemia familial 3, 4, and 5; Hyperinsulinism-hyperammonemia syndrome; Hyperlysinemia; Hypermanganesemia with dystonia, polycythemia and cirrhosis; Hyperornithinemia-hyperammonemia-homocitrullinuria syndrome; Hyperparathyroidism 1 and 2; Hyperparathyroidism, neonatal severe; Hyperphenylalaninemia, bh4-deficient, a, due to partial pts deficiency, BH4-deficient, D, and non-pku; Hyperphosphatasia with mental retardation syndrome 2, 3, and 4; Hypertrichotic osteochondrodysplasia; Hypobetalipoproteinemia, familial, associated with apob32; Hypocalcemia, autosomal dominant 1; Hypocalciuric hypercalcemia, familial, types 1 and 3; Hypochondrogenesis; Hypochromic microcytic anemia with iron overload; Hypoglycemia with deficiency of glycogen synthetase in the liver; Hypogonadotropic hypogonadism 11 with or without anosmia; Hypohidrotic ectodermal dysplasia with immune deficiency; Hypohidrotic X-linked ectodermal dysplasia; Hypokalemic periodic paralysis 1 and 2; Hypomagnesemia 1, intestinal; Hypomagnesemia, seizures, and mental retardation; Hypomyelinating leukodystrophy 7; Hypoplastic left heart syndrome; Atrioventricular septal defect and common atrioventricular junction; Hypospadias 1 and 2, X-linked; Hypothyroidism, congenital, nongoitrous, 1; Hypotrichosis 8 and 12; Hypotrichosis-lymphedema-telangiectasia syndrome; I blood group system; Ichthyosis bullosa of Siemens; Ichthyosis

exfoliativa; Ichthyosis prematurity syndrome; Idiopathic basal ganglia calcification 5; Idiopathic fibrosing alveolitis, chronic form; Dyskeratosis congenita, autosomal dominant, 2 and 5; Idiopathic hypercalcemia of infancy; Immune dysfunction with T-cell inactivation due to calcium entry defect 2; Immunodeficiency 15, 16, 19, 30, 31C, 38, 40, 8, due to defect in cd3-zeta, with hyper IgM type 1 and 2, and X-Linked, with magnesium defect, Epstein-Barr virus infection, and neoplasia; Immunodeficiency-centromeric instability-facial anomalies syndrome 2; Inclusion body myopathy 2 and 3; Nonaka myopathy; Infantile convulsions and paroxysmal choreoathetosis, familial; Infantile cortical hyperostosis; Infantile GM1 gangliosidosis; Infantile hypophosphatasia; Infantile nephronophthisis; Infantile nystagmus, X-linked; Infantile Parkinsonism-dystonia; Infertility associated with multi-tailed spermatozoa and excessive DNA; Insulin resistance; Insulin-resistant diabetes mellitus and acanthosis nigricans; Insulin-dependent diabetes mellitus secretory diarrhea syndrome; Interstitial nephritis, karyomegalic; Intrauterine growth retardation, metaphyseal dysplasia, adrenal hypoplasia congenita, and genital anomalies; Iodotyrosyl coupling defect; IRAK4 deficiency; Iridogoniodysgenesis dominant type and type 1; Iron accumulation in brain; Ischiopatellar dysplasia; Islet cell hyperplasia; Isolated 17,20-lyase deficiency; Isolated lutropin deficiency; Isovaleryl-CoA dehydrogenase deficiency; Jankovic Rivera syndrome; Jervell and Lange-Nielsen syndrome 2; Joubert syndrome 1, 6, 7, 9/15 (digenic), 14, 16, and 17, and Orofaciodigital syndrome xiv; Junctional epidermolysis bullosa gravis of Herlitz; Juvenile GM>1< gangliosidosis; Juvenile polyposis syndrome; Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome; Juvenile retinoschisis; Kabuki make-up syndrome; Kallmann syndrome 1, 2, and 6; Delayed puberty; Kanzaki disease; Karak syndrome; Kartagener syndrome; Kenny-Caffey syndrome type 2; Keppen-Lubinsky syndrome; Keratoconus 1; Keratosis follicularis; Keratosis palmoplantaris striata 1; Kindler syndrome; L-2-hydroxyglutaric aciduria; Larsen syndrome, dominant type; Lattice corneal dystrophy Type III; Leber amaurosis; Zellweger syndrome; Peroxisome biogenesis disorders; Zellweger syndrome spectrum; Leber congenital amaurosis 11, 12, 13, 16, 4, 7, and 9; Leber optic atrophy; Aminoglycoside-induced deafness; Deafness, nonsyndromic sensorineural, mitochondrial; Left ventricular noncompaction 5; Left-right axis malformations; Leigh disease; Mitochondrial short-chain Enoyl-CoA Hydratase 1 deficiency; Leigh syndrome due to mitochondrial complex I deficiency; Leiner disease; Leri Weill dyschondrosteosis; Lethal congenital contracture syndrome 6; Leukocyte adhesion deficiency type I and III; Leukodystrophy, Hypomyelinating, 11 and 6; Leukoencephalopathy with ataxia, with

Brainstem and Spinal Cord Involvement and Lactate Elevation, with vanishing white matter, and progressive, with ovarian failure; Leukonychia totalis; Lewy body dementia; Lichtenstein-Knorr Syndrome; Li-Fraumeni syndrome 1; Lig4 syndrome; Limb-girdle muscular dystrophy, type 1B, 2A, 2B, 2D, C1, C5, C9, C14; Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies, type A14 and B14; Lipase deficiency combined; Lipid proteinosis; Lipodystrophy, familial partial, type 2 and 3; Lissencephaly 1, 2 (X-linked), 3, 6 (with microcephaly), X-linked; Subcortical laminar heterotopia, X-linked; Liver failure acute infantile; Loeys-Dietz syndrome 1, 2, 3; Long QT syndrome 1, 2, 2/9, 2/5, (digenic), 3, 5 and 5, acquired, susceptibility to; Lung cancer; Lymphedema, hereditary, id; Lymphedema, primary, with myelodysplasia; Lymphoproliferative syndrome 1, 1 (X-linked), and 2; Lysosomal acid lipase deficiency; Macrocephaly, macrosomia, facial dysmorphism syndrome; Macular dystrophy, vitelliform, adult-onset; Malignant hyperthermia susceptibility type 1; Malignant lymphoma, non-Hodgkin; Malignant melanoma; Malignant tumor of prostate; Mandibuloacral dysostosis; Mandibuloacral dysplasia with type A or B lipodystrophy, atypical; Mandibulofacial dysostosis, Treacher Collins type, autosomal recessive; Mannose-binding protein deficiency; Maple syrup urine disease type 1A and type 3; Marden Walker like syndrome; Marfan syndrome; Marinesco-Sj\xc3\xb6gren syndrome; Martsolf syndrome; Maturity-onset diabetes of the young, type 1, type 2, type 11, type 3, and type 9; May-Hegglin anomaly; MYH9 related disorders; Sebastian syndrome; McCune-Albright syndrome; Somatotroph adenoma; Sex cord-stromal tumor; Cushing syndrome; McKusick Kaufman syndrome; McLeod neuroacanthocytosis syndrome; Meckel-Gruber syndrome; Medium-chain acyl-coenzyme A dehydrogenase deficiency; Medulloblastoma; Megalencephalic leukoencephalopathy with subcortical cysts 1and 2a; Megalencephaly cutis marmorata telangiectatica congenital; PIK3CA Related Overgrowth Spectrum; Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2; Megaloblastic anemia, thiamine-responsive, with diabetes mellitus and sensorineural deafness; Meier-Gorlin syndromes 1and 4; Melnick-Needles syndrome; Meningioma; Mental retardation, X-linked, 3, 21, 30, and 72; Mental retardation and microcephaly with pontine and cerebellar hypoplasia; Mental retardation X-linked syndromic 5; Mental retardation, anterior maxillary protrusion, and strabismus; Mental retardation, autosomal dominant 12, 13, 15, 24, 3, 30, 4, 5, 6,and 9; Mental retardation, autosomal recessive 15, 44, 46, and 5; Mental retardation, stereotypic movements, epilepsy, and/or cerebral malformations; Mental retardation, syndromic, Claes-Jensen type, X-linked; Mental retardation, X-linked, nonspecific,

syndromic, Hedera type, and syndromic, wu type; Merosin deficient congenital muscular dystrophy; Metachromatic leukodystrophy juvenile, late infantile, and adult types; Metachromatic leukodystrophy; Metatrophic dysplasia; Methemoglobinemia types I and 2; Methionine adenosyltransferase deficiency, autosomal dominant; Methylmalonic acidemia with homocystinuria, ; Methylmalonic aciduria cblB type, ; Methylmalonic aciduria due to methylmalonyl-CoA mutase deficiency; METHYLMALONIC ACIDURIA, mut(0) TYPE; Microcephalic osteodysplastic primordial dwarfism type 2; Microcephaly with or without chorioretinopathy, lymphedema, or mental retardation; Microcephaly, hiatal hernia and nephrotic syndrome; Microcephaly; Hypoplasia of the corpus callosum; Spastic paraplegia 50, autosomal recessive; Global developmental delay; CNS hypomyelination; Brain atrophy; Microcephaly, normal intelligence and immunodeficiency; Microcephaly-capillary malformation syndrome; Microcytic anemia; Microphthalmia syndromic 5, 7, and 9; Microphthalmia, isolated 3, 5, 6, 8, and with coloboma 6; Microspherophakia; Migraine, familial basilar; Miller syndrome; Minicore myopathy with external ophthalmoplegia; Myopathy, congenital with cores; Mitchell-Riley syndrome; mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency; Mitochondrial complex I, II, III, III (nuclear type 2, 4, or 8) deficiency; Mitochondrial DNA depletion syndrome 11, 12 (cardiomyopathic type), 2, 4B (MNGIE type), 8B (MNGIE type); Mitochondrial DNA-depletion syndrome 3 and 7, hepatocerebral types, and 13 (encephalomyopathic type); Mitochondrial phosphate carrier and pyruvate carrier deficiency; Mitochondrial trifunctional protein deficiency; Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency; Miyoshi muscular dystrophy 1; Myopathy, distal, with anterior tibial onset; Mohr-Tranebjaerg syndrome; Molybdenum cofactor deficiency, complementation group A; Mowat-Wilson syndrome; Mucolipidosis III Gamma; Mucopolysaccharidosis type VI, type VI (severe), and type VII; Mucopolysaccharidosis, MPS-I-H/S, MPS-II, MPS-III-A, MPS-III-B, MPS-III-C, MPS-IV-A, MPS-IV-B; Retinitis Pigmentosa 73; Gangliosidosis GM1 type1 (with cardiac involvement) 3; Multicentric osteolysis nephropathy; Multicentric osteolysis, nodulosis and arthropathy; Multiple congenital anomalies; Atrial septal defect 2; Multiple congenital anomalies-hypotonia-seizures syndrome 3; Multiple Cutaneous and Mucosal Venous Malformations; Multiple endocrine neoplasia, types 1and 4; Multiple epiphyseal dysplasia 5 or Dominant; Multiple gastrointestinal atresias; Multiple pterygium syndrome Escobar type; Multiple sulfatase deficiency; Multiple synostoses syndrome 3; Muscle AMP guanine oxidase deficiency; Muscle eye brain disease; Muscular dystrophy, congenital, megaconial type; Myasthenia,

familial infantile, 1; Myasthenic Syndrome, Congenital, 11, associated with acetylcholine receptor deficiency; Myasthenic Syndrome, Congenital, 17, 2A (slow-channel), 4B (fast-channel), and without tubular aggregates; Myeloperoxidase deficiency; MYH-associated polyposis; Endometrial carcinoma; Myocardial infarction 1; Myoclonic dystonia; Myoclonic-Atonic Epilepsy; Myoclonus with epilepsy with ragged red fibers; Myofibrillar myopathy 1 and ZASP-related; Myoglobinuria, acute recurrent, autosomal recessive; Myoneural gastrointestinal encephalopathy syndrome; Cerebellar ataxia infantile with progressive external ophthalmoplegia; Mitochondrial DNA depletion syndrome 4B, MNGIE type; Myopathy, centronuclear, 1, congenital, with excess of muscle spindles, distal, 1, lactic acidosis, and sideroblastic anemia 1, mitochondrial progressive with congenital cataract, hearing loss, and developmental delay, and tubular aggregate, 2; Myopia 6; Myosclerosis, autosomal recessive; Myotonia congenital; Congenital myotonia, autosomal dominant and recessive forms; Nail-patella syndrome; Nance-Horan syndrome; Nanophthalmos 2; Navajo neurohepatopathy; Nemaline myopathy 3 and 9; Neonatal hypotonia; Intellectual disability; Seizures; Delayed speech and language development; Mental retardation, autosomal dominant 31; Neonatal intrahepatic cholestasis caused by citrin deficiency; Nephrogenic diabetes insipidus, Nephrogenic diabetes insipidus, X-linked; Nephrolithiasis/osteoporosis, hypophosphatemic, 2; Nephronophthisis 13, 15 and 4; Infertility; Cerebello-oculo-renal syndrome (nephronophthisis, oculomotor apraxia and cerebellar abnormalities); Nephrotic syndrome, type 3, type 5, with or without ocular abnormalities, type 7, and type 9; Nestor-Guillermo progeria syndrome; Neu-Laxova syndrome 1; Neurodegeneration with brain iron accumulation 4 and 6; Neuroferritinopathy; Neurofibromatosis, type 1and type 2; Neurofibrosarcoma; Neurohypophyseal diabetes insipidus; Neuropathy, Hereditary Sensory, Type IC; Neutral 1 amino acid transport defect; Neutral lipid storage disease with myopathy; Neutrophil immunodeficiency syndrome; Nicolaides-Baraitser syndrome; Niemann-Pick disease type C1, C2, type A, and type C1, adult form; Non-ketotic hyperglycinemia; Noonan syndrome 1 and 4, LEOPARD syndrome 1; Noonan syndrome-like disorder with or without juvenile myelomonocytic leukemia; Normokalemic periodic paralysis, potassium-sensitive; Norum disease; Epilepsy, Hearing Loss, And Mental Retardation Syndrome; Mental Retardation, X-Linked 102 and syndromic 13; Obesity; Ocular albinism, type I; Oculocutaneous albinism type 1B, type 3, and type 4; Oculodentodigital dysplasia; Odontohypophosphatasia; Odontotrichomelic syndrome; Oguchi disease; Oligodontia-colorectal cancer syndrome; Opitz G/BBB syndrome; Optic atrophy 9; Oral-facial-digital

syndrome; Ornithine aminotransferase deficiency; Orofacial cleft 11 and 7, Cleft lip/palate-ectodermal dysplasia syndrome; Orstavik Lindemann Solberg syndrome; Osteoarthritis with mild chondrodysplasia; Osteochondritis dissecans; Osteogenesis imperfecta type 12, type 5, type 7, type 8, type I, type III, with normal sclerae, dominant form, recessive perinatal lethal; Osteopathia striata with cranial sclerosis; Osteopetrosis autosomal dominant type 1 and 2, recessive 4, recessive 1, recessive 6; Osteoporosis with pseudoglioma; Oto-palato-digital syndrome, types I and II; Ovarian dysgenesis 1; Ovarioleukodystrophy; Pachyonychia congenita 4 and type 2; Paget disease of bone, familial; Pallister-Hall syndrome; Palmoplantar keratoderma, nonepidermolytic, focal or diffuse; Pancreatic agenesis and congenital heart disease; Papillon-Lef\xc3\xa8vre syndrome; Paragangliomas 3; Paramyotonia congenita of von Eulenburg; Parathyroid carcinoma; Parkinson disease 14, 15, 19 (juvenile-onset), 2, 20 (early-onset), 6, (autosomal recessive early-onset, and 9; Partial albinism; Partial hypoxanthine-guanine phosphoribosyltransferase deficiency; Patterned dystrophy of retinal pigment epithelium; PC-K6a; Pelizaeus-Merzbacher disease; Pendred syndrome; Peripheral demyelinating neuropathy, central dysmyelination; Hirschsprung disease; Permanent neonatal diabetes mellitus; Diabetes mellitus, permanent neonatal, with neurologic features; Neonatal insulin-dependent diabetes mellitus; Maturity-onset diabetes of the young, type 2; Peroxisome biogenesis disorder 14B, 2A, 4A, 5B, 6A, 7A, and 7B; Perrault syndrome 4; Perry syndrome; Persistent hyperinsulinemic hypoglycemia of infancy; familial hyperinsulinism; Phenotypes; Phenylketonuria; Pheochromocytoma; Hereditary Paraganglioma-Pheochromocytoma Syndromes; Paragangliomas 1; Carcinoid tumor of intestine; Cowden syndrome 3; Phosphoglycerate dehydrogenase deficiency; Phosphoglycerate kinase 1 deficiency; Photosensitive trichothiodystrophy; Phytanic acid storage disease; Pick disease; Pierson syndrome; Pigmentary retinal dystrophy; Pigmented nodular adrenocortical disease, primary, 1; Pilomatrixoma; Pitt-Hopkins syndrome; Pituitary dependent hypercortisolism; Pituitary hormone deficiency, combined 1, 2, 3, and 4; Plasminogen activator inhibitor type 1 deficiency; Plasminogen deficiency, type I; Platelet-type bleeding disorder 15 and 8; Poikiloderma, hereditary fibrosing, with tendon contractures, myopathy, and pulmonary fibrosis; Polycystic kidney disease 2, adult type, and infantile type; Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy; Polyglucosan body myopathy 1 with or without immunodeficiency; Polymicrogyria, asymmetric, bilateral frontoparietal; Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract; Pontocerebellar hypoplasia type 4; Popliteal pterygium syndrome;

Porencephaly 2; Porokeratosis 8, disseminated superficial actinic type; Porphobilinogen synthase deficiency; Porphyria cutanea tarda; Posterior column ataxia with retinitis pigmentosa; Posterior polar cataract type 2; Prader-Willi-like syndrome; Premature ovarian failure 4, 5, 7, and 9; Primary autosomal recessive microcephaly 10, 2, 3, and 5; Primary ciliary dyskinesia 24; Primary dilated cardiomyopathy; Left ventricular noncompaction 6; 4, Left ventricular noncompaction 10; Paroxysmal atrial fibrillation; Primary hyperoxaluria, type I, type, and type III; Primary hypertrophic osteoarthropathy, autosomal recessive 2; Primary hypomagnesemia; Primary open angle glaucoma juvenile onset 1; Primary pulmonary hypertension; Primrose syndrome; Progressive familial heart block type 1B; Progressive familial intrahepatic cholestasis 2 and 3; Progressive intrahepatic cholestasis; Progressive myoclonus epilepsy with ataxia; Progressive pseudorheumatoid dysplasia; Progressive sclerosing poliodystrophy; Prolidase deficiency; Proline dehydrogenase deficiency; Schizophrenia 4; Properdin deficiency, X-linked; Propionic academia; Proprotein convertase 1/3 deficiency; Prostate cancer, hereditary, 2; Protan defect; Proteinuria; Finnish congenital nephrotic syndrome; Proteus syndrome; Breast adenocarcinoma; Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome; Pseudohypoaldosteronism type 1 autosomal dominant and recessive and type 2; Pseudohypoparathyroidism type 1A, Pseudopseudohypoparathyroidism; Pseudoneonatal adrenoleukodystrophy; Pseudoprimary hyperaldosteronism; Pseudoxanthoma elasticum; Generalized arterial calcification of infancy 2; Pseudoxanthoma elasticum-like disorder with multiple coagulation factor deficiency; Psoriasis susceptibility 2; PTEN hamartoma tumor syndrome; Pulmonary arterial hypertension related to hereditary hemorrhagic telangiectasia; Pulmonary Fibrosis And/Or Bone Marrow Failure, Telomere-Related, 1 and 3; Pulmonary hypertension, primary, 1, with hereditary hemorrhagic telangiectasia; Purine-nucleoside phosphorylase deficiency; Pyruvate carboxylase deficiency; Pyruvate dehydrogenase E1-alpha deficiency; Pyruvate kinase deficiency of red cells; Raine syndrome; Rasopathy; Recessive dystrophic epidermolysis bullosa; Nail disorder, nonsyndromic congenital, 8; Reifenstein syndrome; Renal adysplasia; Renal carnitine transport defect; Renal coloboma syndrome; Renal dysplasia; Renal dysplasia, retinal pigmentary dystrophy, cerebellar ataxia and skeletal dysplasia; Renal tubular acidosis, distal, autosomal recessive, with late-onset sensorineural hearing loss, or with hemolytic anemia; Renal tubular acidosis, proximal, with ocular abnormalities and mental retardation; Retinal cone dystrophy 3B; Retinitis pigmentosa; Retinitis pigmentosa 10, 11, 12, 14, 15, 17, and 19; Retinitis pigmentosa 2, 20, 25, 35, 36, 38, 39, 4, 40, 43, 45, 48, 66,

7, 70, 72; Retinoblastoma; Rett disorder; Rhabdoid tumor predisposition syndrome 2; Rhegmatogenous retinal detachment, autosomal dominant; Rhizomelic chondrodysplasia punctata type 2 and type 3; Roberts-SC phocomelia syndrome; Robinow Sorauf syndrome; Robinow syndrome, autosomal recessive, autosomal recessive, with brachy-syn-polydactyly; Rothmund-Thomson syndrome; Rapadilino syndrome; RRM2B-related mitochondrial disease; Rubinstein-Taybi syndrome; Salla disease; Sandhoff disease, adult and infantil types; Sarcoidosis, early-onset; Blau syndrome; Schindler disease, type 1; Schizencephaly; Schizophrenia 15; Schneckenbecken dysplasia; Schwannomatosis 2; Schwartz Jampel syndrome type 1; Sclerocornea, autosomal recessive; Sclerosteosis; Secondary hypothyroidism; Segawa syndrome, autosomal recessive; Senior-Loken syndrome 4 and 5, ; Sensory ataxic neuropathy, dysarthria, and ophthalmoparesis; Sepiapterin reductase deficiency; SeSAME syndrome; Severe combined immunodeficiency due to ADA deficiency, with microcephaly, growth retardation, and sensitivity to ionizing radiation, atypical, autosomal recessive, T cell-negative, B cell-positive, NK cell-negative of NK-positive; Severe congenital neutropenia; Severe congenital neutropenia 3, autosomal recessive or dominant; Severe congenital neutropenia and 6, autosomal recessive; Severe myoclonic epilepsy in infancy; Generalized epilepsy with febrile seizures plus, types 1 and 2; Severe X-linked myotubular myopathy; Short QT syndrome 3; Short stature with nonspecific skeletal abnormalities; Short stature, auditory canal atresia, mandibular hypoplasia, skeletal abnormalities; Short stature, onychodysplasia, facial dysmorphism, and hypotrichosis; Primordial dwarfism; Short-rib thoracic dysplasia 11 or 3 with or without polydactyly; Sialidosis type I and II; Silver spastic paraplegia syndrome; Slowed nerve conduction velocity, autosomal dominant; Smith-Lemli-Opitz syndrome; Snyder Robinson syndrome; Somatotroph adenoma; Prolactinoma; familial, Pituitary adenoma predisposition; Sotos syndrome 1 or 2; Spastic ataxia 5, autosomal recessive, Charlevoix-Saguenay type, 1,10, or 11, autosomal recessive; Amyotrophic lateral sclerosis type 5; Spastic paraplegia 15, 2, 3, 35, 39, 4, autosomal dominant, 55, autosomal recessive, and 5A; Bile acid synthesis defect, congenital, 3; Spermatogenic failure 11, 3, and 8; Spherocytosis types 4 and 5; Spheroid body myopathy; Spinal muscular atrophy, lower extremity predominant 2, autosomal dominant; Spinal muscular atrophy, type II; Spinocerebellar ataxia 14, 21, 35, 40,and 6; Spinocerebellar ataxia autosomal recessive 1 and 16; Splenic hypoplasia; Spondylocarpotarsal synostosis syndrome; Spondylocheirodysplasia, Ehlers-Danlos syndrome-like, with immune dysregulation, Aggrecan type, with congenital joint dislocations, short limb-hand type,

Sedaghatian type, with cone-rod dystrophy, and Kozlowski type; Parastremmatic dwarfism; Stargardt disease 1; Cone-rod dystrophy 3; Stickler syndrome type 1; Kniest dysplasia; Stickler syndrome, types 1(nonsyndromic ocular) and 4; Sting-associated vasculopathy, infantile-onset; Stormorken syndrome; Sturge-Weber syndrome, Capillary malformations, congenital, 1; Succinyl-CoA acetoacetate transferase deficiency; Sucrase-isomaltase deficiency; Sudden infant death syndrome; Sulfite oxidase deficiency, isolated; Supravalvar aortic stenosis; Surfactant metabolism dysfunction, pulmonary, 2 and 3; Symphalangism, proximal, 1b; Syndactyly Cenani Lenz type; Syndactyly type 3; Syndromic X-linked mental retardation 16; Talipes equinovarus; Tangier disease; TARP syndrome; Tay-Sachs disease, B1 variant, Gm2-gangliosidosis (adult), Gm2-gangliosidosis (adult-onset); Temtamy syndrome; Tenorio Syndrome; Terminal osseous dysplasia; Testosterone 17-beta-dehydrogenase deficiency; Tetraamelia, autosomal recessive; Tetralogy of Fallot; Hypoplastic left heart syndrome 2; Truncus arteriosus; Malformation of the heart and great vessels; Ventricular septal defect 1; Thiel-Behnke corneal dystrophy; Thoracic aortic aneurysms and aortic dissections; Marfanoid habitus; Three M syndrome 2; Thrombocytopenia, platelet dysfunction, hemolysis, and imbalanced globin synthesis; Thrombocytopenia, X-linked; Thrombophilia, hereditary, due to protein C deficiency, autosomal dominant and recessive; Thyroid agenesis; Thyroid cancer, follicular; Thyroid hormone metabolism, abnormal; Thyroid hormone resistance, generalized, autosomal dominant; Thyrotoxic periodic paralysis and Thyrotoxic periodic paralysis 2; Thyrotropin-releasing hormone resistance, generalized; Timothy syndrome; TNF receptor-associated periodic fever syndrome (TRAPS); Tooth agenesis, selective, 3 and 4; Torsades de pointes; Townes-Brocks-branchiootorenal-like syndrome; Transient bullous dermolysis of the newborn; Treacher collins syndrome 1; Trichomegaly with mental retardation, dwarfism and pigmentary degeneration of retina; Trichorhinophalangeal dysplasia type I; Trichorhinophalangeal syndrome type 3; Trimethylaminuria; Tuberous sclerosis syndrome; Lymphangiomyomatosis; Tuberous sclerosis 1 and 2; Tyrosinase-negative oculocutaneous albinism; Tyrosinase-positive oculocutaneous albinism; Tyrosinemia type I; UDPglucose-4-epimerase deficiency; Ullrich congenital muscular dystrophy; Ulna and fibula absence of with severe limb deficiency; Upshaw-Schulman syndrome; Urocanate hydratase deficiency; Usher syndrome, types 1, 1B, 1D, 1G, 2A, 2C, and 2D; Retinitis pigmentosa 39; UV-sensitive syndrome; Van der Woude syndrome; Van Maldergem syndrome 2; Hennekam lymphangiectasia-lymphedema syndrome 2; Variegate porphyria; Ventriculomegaly with

cystic kidney disease; Verheij syndrome; Very long chain acyl-CoA dehydrogenase deficiency; Vesicoureteral reflux 8; Visceral heterotaxy 5, autosomal; Visceral myopathy; Vitamin D-dependent rickets, types 1and 2; Vitelliform dystrophy ; von Willebrand disease type 2M and type 3; Waardenburg syndrome type 1, 4C, and 2E (with neurologic involvement); Klein-Waardenberg syndrome; Walker-Warburg congenital muscular dystrophy; Warburg micro syndrome 2 and 4; Warts, hypogammaglobulinemia, infections, and myelokathexis; Weaver syndrome; Weill-Marchesani syndrome 1 and 3; Weill-Marchesani-like syndrome; Weissenbacher-Zweymuller syndrome; Werdnig-Hoffmann disease; Charcot-Marie-Tooth disease; Werner syndrome; WFS1-Related Disorders; Wiedemann-Steiner syndrome; Wilson disease; Wolfram-like syndrome, autosomal dominant; Worth disease; Van Buchem disease type 2; Xeroderma pigmentosum, complementation group b, group D, group E, and group G; X-linked agammaglobulinemia; X-linked hereditary motor and sensory neuropathy; X-linked ichthyosis with steryl-sulfatase deficiency; X-linked periventricular heterotopia; Oto-palato-digital syndrome, type I; X-linked severe combined immunodeficiency; Zimmermann-Laband syndrome and Zimmermann-Laband syndrome 2; and Zonular pulverulent cataract 3.

[1048] The target nucleotide sequence may comprise a target sequence (e.g., a point mutation) associated with a disease, disorder, or condition. The target sequence may comprise a T to C (or A to G) point mutation associated with a disease, disorder, or condition, and wherein the deamination of the mutant C base results in mismatch repair-mediated correction to a sequence that is not associated with a disease, disorder, or condition. The target sequence may comprise a G to A (or C to T) point mutation associated with a disease, disorder, or condition, and wherein the deamination of the mutant A base results in mismatch repair-mediated correction to a sequence that is not associated with a disease, disorder, or condition. The target sequence may encode a protein, and where the point mutation is in a codon and results in a change in the amino acid encoded by the mutant codon as compared to a wild-type codon. The target sequence may also be at a splice site, and the point mutation results in a change in the splicing of an mRNA transcript as compared to a wild-type transcript. In addition, the target may be at a non-coding sequence of a gene, such as a promoter, and the point mutation results in increased or decreased expression of the gene.

[1049] Thus, in some aspects, the deamination of a mutant C results in a change of the amino acid encoded by the mutant codon, which in some cases can result in the expression of a wild-type amino acid. In other aspects, the deamination of a mutant A results in a change of

the amino acid encoded by the mutant codon, which in some cases can result in the expression of a wild-type amino acid.

[1050] The methods described herein involving contacting a cell with a composition or rAAV particle can occur *in vitro, ex vivo,* or *in vivo*. In certain embodiments, the step of contacting occurs in a subject. In certain embodiments, the subject has been diagnosed with a disease, disorder, or condition.

[1051] In some embodiments, the methods disclosed herein involve contacting a mammalian cell with a composition or rAAV particle. In particular embodiments, the methods involve contacting a retinal cell, cortical cell or cerebellar cell.

[1052] The split Cas9 protein or split prime editor delivered using the methods described herein preferably have comparable activity compared to the original Cas9 protein or prime editor (*i.e.*, unsplit protein delivered to a cell or expressed in a cell as a whole). For example, the split Cas9 protein or split prime editor retains at least 50% (*e.g.*, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%) of the activity of the original Cas9 protein or prime editor. In some embodiments, the split Cas9 protein or split prime editor is more active (*e.g.*, 2-fold, 5-fold, 10-fold, 100-fold, 1000-fold, or more) than that of an original Cas9 protein or prime editor.

[1053] The compositions described herein may be administered to a subject in need thereof in a therapeutically effective amount to treat and/or prevent a disease or disorder the subject is suffering from. Any disease or disorder that maybe treated and/or prevented using CRISPR/Cas9-based genome-editing technology may be treated by the split Cas9 protein or the split prime editor described herein. It is to be understood that, if the nucleotide sequences encoding the split Cas9 protein or the prime editor does not further encode a gRNA, a separate nucleic acid vector encoding the gRNA may be administered together with the compositions described herein.

[1054] Exemplary suitable diseases, disorders or conditions include, without limitation the disease or disorder is selected from the group consisting of: cystic fibrosis, phenylketonuria, epidermolytic hyperkeratosis (EHK), chronic obstructive pulmonary disease (COPD), Charcot-Marie-Toot disease type 4J, neuroblastoma (NB), von Willebrand disease (vWD), myotonia congenital, hereditary renal amyloidosis, dilated cardiomyopathy, hereditary lymphedema, familial Alzheimer's disease, prion disease, chronic infantile neurologic cutaneous articular syndrome (CINCA), congenital deafness, Niemann-Pick disease type C

(NPC) disease, and desmin-related myopathy (DRM). In particular embodiments, the disease or condition is Niemann-Pick disease type C (NPC) disease.

[1055] In some embodiments, the disease, disorder or condition is associated with a point mutation in an *NPC* gene, a *DNMT1* gene, a *PCSK9* gene, or a *TMC1* gene. In certain embodiments, the point mutation is a T3182C mutation in *NPC*, which results in an I1061T amino acid substitution.

[1056] In certain embodiments, the point mutation is an A545G mutation in *TMC1*, which results in a Y182C amino acid substitution. *TMC1* encodes a protein that forms mechanosensitive ion channels in sensory hair cells of the inner ear and is required for normal auditory function. The Y182C amino acid substitution is associated with congenital deafness.

[1057] In some embodiments, the disease, disorder or condition is associated with a point mutation that generates a stop codon, for example, a premature stop codon within the coding region of a gene.

[1058] Additional exemplary diseases, disorders and conditions include cystic fibrosis (see, *e.g.*, Schwank *et al.*, Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell stem cell.* **2013**; 13: 653-658; and Wu et. al., Correction of a genetic disease in mouse via use of CRISPR-Cas9. *Cell stem cell.* **2013;** 13: 659-662, neither of which uses a deaminase fusion protein to correct the genetic defect); phenylketonuria – *e.g.*, phenylalanine to serine mutation at position 835 (mouse) or 240 (human) or a homologous residue in phenylalanine hydroxylase gene (T>C mutation) – see, *e.g.*, McDonald *et al.*, *Genomics.* **1997;** 39:402-405; Bernard-Soulier syndrome (BSS) – *e.g.*, phenylalanine to serine mutation at position 55 or a homologous residue, or cysteine to arginine at residue 24 or a homologous residue in the platelet membrane glycoprotein IX (T>C mutation) – see, *e.g.*, Noris *et al.*, *British Journal of Haematology.* **1997;** 97: 312-320, and Ali *et al.*, *Hematol.* **2014;** 93: 381-384; epidermolytic hyperkeratosis (EHK) – *e.g.*, leucine to proline mutation at position 160 or 161 (if counting the initiator methionine) or a homologous residue in keratin 1 (T>C mutation) – see, *e.g.*, Chipev *et al.*, *Cell.* **1992;** 70: 821-828, see also accession number P04264 in the UNIPROT database at www[dot]uniprot[dot]org; chronic obstructive pulmonary disease (COPD) – *e.g.*, leucine to proline mutation at position 54 or 55 (if counting the initiator methionine) or a homologous residue in the processed form of α_1-antitrypsin or residue 78 in the unprocessed form or a homologous residue (T>C mutation) – see, *e.g.*, Poller *et al.*, *Genomics.* **1993;** 17: 740-743,

see also accession number P01011 in the UNIPROT database; Charcot-Marie-Toot disease type 4J – *e.g.*, isoleucine to threonine mutation at position 41 or a homologous residue in FIG4 (T>C mutation) – see, *e.g.*, Lenk *et al.*, PLoS Genetics. 2011; 7: e1002104; neuroblastoma (NB) – *e.g.*, leucine to proline mutation at position 197 or a homologous residue in Caspase-9 (T>C mutation) – see, *e.g.*, Kundu *et al.*, *3 Biotech.* **2013, 3:225-234**; von Willebrand disease (vWD) – *e.g.*, cysteine to arginine mutation at position 509 or a homologous residue in the processed form of von Willebrand factor, or at position 1272 or a homologous residue in the unprocessed form of von Willebrand factor (T>C mutation) – see, *e.g.*, Lavergne *et al.*, *Br. J. Haematol.* **1992**, see also accession number P04275 in the UNIPROT database**;** 82: 66-72; myotonia congenital – *e.g.*, cysteine to arginine mutation at position 277 or a homologous residue in the muscle chloride channel gene CLCN1 (T>C mutation) – see, *e.g.*, Weinberger *et al.*, *The J. of Physiology.* **2012;** 590: 3449-3464; hereditary renal amyloidosis – *e.g.*, stop codon to arginine mutation at position 78 or a homologous residue in the processed form of apolipoprotein AII or at position 101 or a homologous residue in the unprocessed form (T>C mutation) – see, *e.g.*, Yazaki *et al.*, *Kidney Int.* **2003;** 64: 11-16; dilated cardiomyopathy (DCM) – *e.g.*, tryptophan to Arginine mutation at position 148 or a homologous residue in the FOXD4 gene (T>C mutation), see, *e.g.*, Minoretti et. al., *Int. J. of Mol. Med.* **2007;** 19: 369-372; hereditary lymphedema – *e.g.*, histidine to arginine mutation at position 1035 or a homologous residue in VEGFR3 tyrosine kinase (A>G mutation), see, *e.g.*, Irrthum *et al.*, *Am. J. Hum. Genet.* **2000;** 67: 295-301; familial Alzheimer's disease – *e.g.*, isoleucine to valine mutation at position 143 or a homologous residue in presenilin1 (A>G mutation), see, *e.g.*, Gallo et. al., *J. Alzheimer's disease.* **2011;** 25: 425-431; Prion disease – *e.g.*, methionine to valine mutation at position 129 or a homologous residue in prion protein (A>G mutation) – see, *e.g.*, Lewis et. al., *J. of General Virology.* **2006;** 87: 2443-2449; chronic infantile neurologic cutaneous articular syndrome (CINCA) – *e.g.*, Tyrosine to Cysteine mutation at position 570 or a homologous residue in cryopyrin (A>G mutation) – see, *e.g.*, Fujisawa et. al. *Blood.* **2007;** 109: 2903-2911; and desmin-related myopathy (DRM) – *e.g.*, arginine to glycine mutation at position 120 or a homologous residue in αβ crystallin (A>G mutation) – see, *e.g.*, Kumar *et al.*, *J. Biol. Chem.* **1999;** 274: 24137-24141. The entire contents of all references and database entries is incorporated herein by reference.

Trinucleotide repeat expansion disease

[1059] Trinucleotide repeat expansion is associated with a number of human diseases, including Huntington's Disease, Fragile X syndrome, and Friedreich's ataxia. The most common trinucleotide repeat contains CAG triplets, though GAA triplets (Friedreich's ataxia) and CGG triplets (Fragile X syndrome) also occur. Inheriting a predisposition to expansion, or acquiring an already expanded parental allele, increases the likelihood of acquiring the disease. Pathogenic expansions of trinucleotide repeats could hypothetically be corrected using prime editing.

[1060] A region upstream of the repeat region can be nicked by an RNA-guided nuclease, then used to prime synthesis of a new DNA strand that contains a healthy number of repeats (which depends on the particular gene and disease), in accordance with the general mechanism outlined in FIG. 1G or FIG. 22. After the repeat sequence, a short stretch of homology is added that matches the identity of the sequence adjacent to the other end of the repeat (red strand). Invasion of the newly synthesized strand by the TPRT system, and subsequent replacement of the endogenous DNA with the newly synthesized flap, leads to a contracted repeat allele. The term "contracted" refers to a shortening of the length of the nucleotide repeat region, thereby resulting in repairing the trinucleotide repeat region.

[1061] The prime editing system or prime editing (PE) system described herein may be used to contract trinucleotide repeat mutations (or "triplet expansion diseases") to treating conditions such as Huntington's disease and other trinucleotide repeat disorders. Trinucleotide repeat expansion disorders are complex, progressive disorders that involve developmental neurobiology and often affect cognition as well as sensori-motor functions. The disorders show genetic anticipation (i.e. increased severity with each generation). The DNA expansions or contractions usually happen meiotically (i.e. during the time of gametogenesis, or early in embryonic development), and often have sex-bias meaning that some genes expand only when inherited through the female, others only through the male. In humans, trinucleotide repeat expansion disorders can cause gene silencing at either the transcriptional or translational level, which essentially knocks out gene function. Alternatively, trinucleotide repeat expansion disorders can cause altered proteins generated with large repetitive amino acid sequences that either abrogate or change protein function, often in a dominant-negative manner (e.g. poly-glutamine diseases).

[1062] Without wishing to be bound by theory, triplet expansion is caused by slippage during DNA replication or during DNA repair synthesis. Because the tandem repeats have identical sequence to one another, base pairing between two DNA strands can take place at multiple

points along the sequence. This may lead to the formation of "loop out" structures during DNA replication or DNA repair synthesis. This may lead to repeated copying of the repeated sequence, expanding the number of repeats. Additional mechanisms involving hybrid RNA:DNA intermediates have been proposed. Prime editing may be used to reduce or eliminate these triplet expansion regions by deletion one or more or the offending repeat codon triplets. In an embodiment of this use, FIG. 23, provides a schematic of a PEgRNA design for contracting or reducing trinucleotide repeat sequences with prime editing.

[1063] Prime editing may be implemented to contract triplet expansion regions by nicking a region upstream of the triplet repeat region with the prime editor comprising a PEgRNA appropriated targeted to the cut site. The prime editor then synthesizes a new DNA strand (ssDNA flap) based on the PEgRNA as a template (i.e., the edit template thereof) that codes for a healthy number of triplet repeats (which depends on the particular gene and disease). The newly synthesized ssDNA strand comprising the healthy triplet repeat sequence also is synthesized to include a short stretch of homology (i.e., the homology arm) that matches the sequence adjacent to the other end of the repeat (red strand). Invasion of the newly synthesized strand, and subsequent replacement of the endogenous DNA with the newly synthesized ssDNA flap, leads to a contracted repeat allele.

[1064] Depending on the particular trinucleotide expansion disorder, the defect-inducing triplet expansions may occur in "trinucleotide repeat expansion proteins." Trinucleotide repeat expansion proteins are a diverse set of proteins associated with susceptibility for developing a trinucleotide repeat expansion disorder, the presence of a trinucleotide repeat expansion disorder, the severity of a trinucleotide repeat expansion disorder or any combination thereof. Trinucleotide repeat expansion disorders are divided into two categories determined by the type of repeat. The most common repeat is the triplet CAG, which, when present in the coding region of a gene, codes for the amino acid glutamine (Q). Therefore, these disorders are referred to as the polyglutamine (polyQ) disorders and comprise the following diseases: Huntington Disease (HD); Spinobulbar Muscular Atrophy (SBMA); Spinocerebellar Ataxias (SCA types 1, 2, 3, 6, 7, and 17); and Dentatorubro-Pallidoluysian Atrophy (DRPLA). The remaining trinucleotide repeat expansion disorders either do not involve the CAG triplet or the CAG triplet is not in the coding region of the gene and are, therefore, referred to as the non-polyglutamine disorders. The non-polyglutamine disorders comprise Fragile X Syndrome (FRAXA); Fragile XE Mental Retardation (FRAXE);

Friedreich Ataxia (FRDA); Myotonic Dystrophy (DM); and Spinocerebellar Ataxias (SCA types 8, and 12).

[1065] The proteins associated with trinucleotide repeat expansion disorders can be selected based on an experimental association of the protein associated with a trinucleotide repeat expansion disorder to a trinucleotide repeat expansion disorder. For example, the production rate or circulating concentration of a protein associated with a trinucleotide repeat expansion disorder may be elevated or depressed in a population having a trinucleotide repeat expansion disorder relative to a population lacking the trinucleotide repeat expansion disorder. Differences in protein levels may be assessed using proteomic techniques including but not limited to Western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA), and mass spectrometry. Alternatively, the proteins associated with trinucleotide repeat expansion disorders may be identified by obtaining gene expression profiles of the genes encoding the proteins using genomic techniques including but not limited to DNA microarray analysis, serial analysis of gene expression (SAGE), and quantitative real-time polymerase chain reaction (Q-PCR).

[1066] Non-limiting examples of proteins associated with trinucleotide repeat expansion disorders which can be corrected by prime editing include AR (androgen receptor), FMR1 (fragile X mental retardation 1), HTT (huntingtin), DMPK (dystrophia myotonica-protein kinase), FXN (frataxin), ATXN2 (ataxin 2), ATN1 (atrophin 1), FEN1 (flap structure-specific endonuclease 1), TNRC6A (trinucleotide repeat containing 6A), PABPN1 (poly(A) binding protein, nuclear 1), JPH3 (junctophilin 3), MED15 (mediator complex subunit 15), ATXN1 (ataxin 1), ATXN3 (ataxin 3), TBP (TATA box binding protein), CACNA1A (calcium channel, voltage-dependent, P/Q type, alpha 1A subunit), ATXN80S (ATXN8 opposite strand (non-protein coding)), PPP2R2B (protein phosphatase 2, regulatory subunit B, beta), ATXN7 (ataxin 7), TNRC6B (trinucleotide repeat containing 6B), TNRC6C (trinucleotide repeat containing 6C), CELF3 (CUGBP, Elav-like family member 3), MAB21L1 (mab-21-like 1 (C. elegans)), MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)), TMEM185A (transmembrane protein 185A), SIX5 (SIX homeobox 5), CNPY3 (canopy 3 homolog (zebrafish)), FRAXE (fragile site, folic acid type, rare, fra(X)(q28) E), GNB2 (guanine nucleotide binding protein (G protein), beta polypeptide 2), RPL14 (ribosomal protein L14), ATXN8 (ataxin 8), INSR (insulin receptor), TTR (transthyretin), EP400 (E1A binding protein p400), GIGYF2 (GRB10 interacting GYF protein 2), OGG1 (8-oxoguanine DNA glycosylase), STC1 (stanniocalcin 1), CNDP1 (carnosine dipeptidase 1

(metallopeptidase M20 family)), C10orf2 (chromosome 10 open reading frame 2), MAML3 mastermind-like 3 (Drosophila), DKC1 (dyskeratosis congenita 1, dyskerin), PAXIP1 (PAX interacting (with transcription-activation domain) protein 1), CASK (calcium/calmodulin-dependent serine protein kinase (MAGUK family)), MAPT (microtubule-associated protein tau), SP1 (Sp1 transcription factor), POLG (polymerase (DNA directed), gamma), AFF2 (AF4/FMR2 family, member 2), THBS1 (thrombospondin 1), TP53 (tumor protein p53), ESR1 (estrogen receptor 1), CGGBP1 (CGG triplet repeat binding protein 1), ABT1 (activator of basal transcription 1), KLK3 (kallikrein-related peptidase 3), PRNP (prion protein), JUN (jun oncogene), KCNN3 (potassium intermediate/small conductance calcium-activated channel, subfamily N, member 3), BAX (BCL2-associated X protein), FRAXA (fragile site, folic acid type, rare, fra(X)(q27.3) A (macroorchidism, mental retardation)), KBTBD10 (kelch repeat and BTB (POZ) domain containing 10), MBNL1 (muscleblind-like (Drosophila)), RAD51 (RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)), NCOA3 (nuclear receptor coactivator 3), ERDA1 (expanded repeat domain, CAG/CTG 1), TSC1 (tuberous sclerosis 1), COMP (cartilage oligomeric matrix protein), GCLC (glutamate-cysteine ligase, catalytic subunit), RRAD (Ras-related associated with diabetes), MSH3 (mutS homolog 3 (E. coli)), DRD2 (dopamine receptor D2), CD44 (CD44 molecule (Indian blood group)), CTCF (CCCTC-binding factor (zinc finger protein)), CCND1 (cyclin D1), CLSPN (claspin homolog (Xenopus laevis)), MEF2A (myocyte enhancer factor 2A), PTPRU (protein tyrosine phosphatase, receptor type, U), GAPDH (glyceraldehyde-3-phosphate dehydrogenase), TRIM22 (tripartite motif-containing 22), WT1 (Wilms tumor 1), AHR (aryl hydrocarbon receptor), GPX1 (glutathione peroxidase 1), TPMT (thiopurine S-methyltransferase), NDP (Norrie disease (pseudoglioma)), ARX (aristaless related homeobox), MUS81 (MUS81 endonuclease homolog (S. cerevisiae)), TYR (tyrosinase (oculocutaneous albinism IA)), EGR1 (early growth response 1), UNG (uracil-DNA glycosylase), NUMBL (numb homolog (Drosophila)-like), FABP2 (fatty acid binding protein 2, intestinal), EN2 (engrailed homeobox 2), CRYGC (crystallin, gamma C), SRP14 (signal recognition particle 14 kDa (homologous Alu RNA binding protein)), CRYGB (crystallin, gamma B), PDCD1 (programmed cell death 1), HOXA1 (homeobox A1), ATXN2L (ataxin 2-like), PMS2 (PMS2 postmeiotic segregation increased 2 (S. cerevisiae)), GLA (galactosidase, alpha), CBL (Cas-Br-M (murine) ecotropic retroviral transforming sequence), FTH1 (ferritin, heavy polypeptide 1), IL12RB2 (interleukin 12 receptor, beta 2), OTX2 (orthodenticle homeobox 2), HOXA5 (homeobox A5), POLG2 (polymerase (DNA directed),

gamma 2, accessory subunit), DLX2 (distal-less homeobox 2), SIRPA (signal-regulatory protein alpha), OTX1 (orthodenticle homeobox 1), AHRR (aryl-hydrocarbon receptor repressor), MANF (mesencephalic astrocyte-derived neurotrophic factor), TMEM158 (transmembrane protein 158 (gene/pseudogene)), and ENSG00000078687.

[1067] In a particular aspect, the instant disclosure provides TPRT-based methods for the treatment of a subject diagnosed with an expansion repeat disorder (also known as a repeat expansion disorder or a trinucleotide repeat disorder). Expansion repeat disorders occur when microsatellite repeats expand beyond a threshold length. Currently, at least 30 genetic diseases are believed to be caused by repeat expansions. Scientific understanding of this diverse group of disorders came to lights in the early 1990's with the discovery that trinucleotide repeats underlie several major inherited conditions, including Fragile X, Spinal and Bulbar Muscular Atrophy, Myotonic Dystrophy, and Huntington's disease (Nelson et al, "The unstable repeats – three evolving faces of neurological disease," *Neuron*, March 6, 2013, Vol.77; 825-843, which is incorporated herein by reference), as well as Haw River Syndrome, Jacobsen Syndrome, Dentatorubral-pallidoluysian atrophy (DRPLA), Machado-Joseph disease, Synpolydactyly (SPD II), Hand-foot genital syndrome (HFGS), Cleidocranial dysplasia (CCD), Holoprosencephaly disorder (HPE), Congenital central hypventilation syndrome (CCHS), ARX-nonsyndromic X-linked mental retardation (XLMR), and Oculopharyngeal muscular dystrophy (OPMD) (see . Microsatellite repeat instability was found to be a hallmark of these conditions, as was anticipation – the phenomenon in which repeat expansion can occur with each successive generation, which leads to a more severe phenotype and earlier age of onset in the offspring. Repeat expansions are believed to cause diseases via several different mechanisms. Namely, expansions may interfere with cellular functioning at the level of the gene, the mRNA transcript, and/or the encoded protein. In some conditions, mutations act via a loss-of-function mechanism by silencing repeat-containing genes. In others, disease results from gain-of-function mechanisms, whereby either the mRNA transcript or protein takes on new, aberrant functions.

[1068] In one embodiment, a method of treating a trinucleotide repeat disorder is depicted in FIG. 23. In general, the approach involves using TPRT genome editing (i.e., prime editing) in combination with an extended gRNA that comprises a region that encodes a desired and healthy replacement trinucleotide repeat sequence that is intended to replace the endogenous diseased trinucleotide repeat sequence through the mechanism of the prime editing process. A schematic of an exemplary gRNA design for contracting trinucleotide repeat sequences and

trinucleotide repeat contraction with TPRT genome editing (i.e., prime editing) is shown in FIG. 23.

Prion disease

[1069] Prime editing can also be used to prevent or halt the progression of prion disease through the installation of one or more protective mutations into prion proteins (PRNP) which become misfolded during the course of disease. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce inflammatory response.

[1070] In humans, prion disease includes Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, and Kuru. In animals, prion disease includes Bovine Spongiform Encephalopathy (BSE or "mad cow disease"), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy. Prime editing may be used to install protective point mutations into a prion protein in order to prevent or halt the progression of any one of these prion diseases.

[1071] Classic CJD is a human prion disease. It is a neurodegenerative disorder with characteristic clinical and diagnostic features. This disease is rapidly progressive and always fatal. Infection with this disease leads to death usually within 1 year of onset of illness. CJD is a rapidly progressive, invariably fatal neurodegenerative disorder believed to be caused by an abnormal isoform of a cellular glycoprotein known as the prion protein. CJD occurs worldwide and the estimated annual incidence in many countries, including the United States, has been reported to be about one case per million population. The vast majority of CJD patients usually die within 1 year of illness onset. CJD is classified as a transmissible spongiform encephalopathy (TSE) along with other prion diseases that occur in humans and animals. In about 85% of patients, CJD occurs as a sporadic disease with no recognizable pattern of transmission. A smaller proportion of patients (5 to 15%) develop CJD because of inherited mutations of the prion protein gene. These inherited forms include Gerstmann-Straussler-Scheinker syndrome and fatal familial insomnia. No treatment is currently known for CJD.

[1072] Variant Creutzfeldt-Jakob disease (vCJD) is a prion disease that was first described in 1996 in the United Kingdom. There is now strong scientific evidence that the agent responsible for the outbreak of prion disease in cows, bovine spongiform encephalopathy (BSE or 'mad cow' disease), is the same agent responsible for the outbreak of vCJD in humans. Variant CJD (vCJD) is not the same disease as classic CJD (often simply called CJD). It has different clinical and pathologic characteristics from classic CJD. Each disease also has a particular genetic profile of the prion protein gene. Both disorders are invariably fatal brain diseases with unusually long incubation periods measured in years, and are caused by an unconventional transmissible agent called a prion. No treatment is currently known for vCJD.

[1073] BSE (bovine spongiform encephalopathy or "mad cow disease") is a progressive neurological disorder of cattle that results from infection by an unusual transmissible agent called a prion. The nature of the transmissible agent is not well understood. Currently, the most accepted theory is that the agent is a modified form of a normal protein known as prion protein. For reasons that are not yet understood, the normal prion protein changes into a pathogenic (harmful) form that then damages the central nervous system of cattle. There is increasing evidence that there are different strains of BSE: the typical or classic BSE strain responsible for the outbreak in the United Kingdom and two atypical strains (H and L strains). No treatment is currently known for BSE.

[1074] Chronic wasting disease (CWD) is a prion disease that affects deer, elk, reindeer, sika deer and moose. It has been found in some areas of North America, including Canada and the United States, Norway and South Korea. It may take over a year before an infected animal develops symptoms, which can include drastic weight loss (wasting), stumbling, listlessness and other neurologic symptoms. CWD can affect animals of all ages and some infected animals may die without ever developing the disease. CWD is fatal to animals and there are no treatments or vaccines.

[1075] The causative agents of TSEs are believed to be prions. The term "prions" refers to abnormal, pathogenic agents that are transmissible and are able to induce abnormal folding of specific normal cellular proteins called prion proteins that are found most abundantly in the brain. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. Prion diseases are usually rapidly progressive and always fatal.

[1076] As used herein, the term "prion" shall mean an infectious particle known to cause diseases (spongiform encephalopathies) in humans and animals. The term "prion" is a contraction of the words "protein" and "infection" and the particles are comprised largely if not exclusively of $PRNP^{Sc}$ molecules encoded by a PRNP gene which expresses $PRNP^{C}$ which changes conformation to become $PRNP^{Sc}$. Prions are distinct from bacteria, viruses and viroids. Known prions include those which infect animals to cause scrapie, a transmissible, degenerative disease of the nervous system of sheep and goats as well as bovine spongiform encephalopathies (BSE) or mad cow disease and feline spongiform encephalopathies of cats. Four prion diseases, as discussed above, known to affect humans are (1) kuru, (2) Creutzfeldt-Jakob Disease (CJD), (3) Gerstmann-Strassler-Scheinker Disease (GSS), and (4) fatal familial insomnia (FFI). As used herein prion includes all forms of prions causing all or any of these diseases or others in any animals used--and in particular in humans and in domesticated farm animals.

[1077] In general, and without wishing to be bound by theory, prior diseases are caused by misfolding of prion proteins. Such diseases—often called deposition diseases—the misfolding of the prion proteins can be accounted for as follows. If A is the normally synthesized gene product that carries out an intended physiologic role in a monomeric or oligomeric state, A* is a conformationally activated form of A that is competent to undergo a dramatic conformational change, B is the conformationally altered state that prefers multimeric assemblies (i.e., the misfolded form which forms depositions) and B_n is the multimeric material that is pathogenic and relatively difficult to recycle. For the prion diseases, $PRNP^{C}$ and $PRNP^{Sc}$ correspond to states A and B_n where A is largely helical and monomeric and B_n is β-rich and multimeric.

[1078] It is known that certain mutations in prion proteins can be associated with increased risk of prior disease. Conversely, certain mutations in prion proteins can be protective in nature. See Bagynszky et al., "Characterization of mutations in PRNP (prion) gene and their possible roles in neurodegenerative diseases," *Neuropsychiatr Dis Treat.*, 2018; 14: 2067-2085, the contents of which are incorporated herein by reference.

[1079] PRNP (NCBI RefSeq No. NP_000302.1 (SEQ ID NO: 291))—the human prion protein--is encoded by a 16 kb long gene, located on chromosome 20 (4686151-4701588). It contains two exons, and the exon 2 carries the open reading frame which encodes the 253 amino acid (AA) long PrP protein. Exon 1 is a noncoding exon, which may serve as transcriptional initiation site. The post-translational modifications result in the removal of the

first 22 AA N-terminal fragment (NTF) and the last 23 AA C-terminal fragment (CTF). The NTF is cleaved after PrP transport to the endoplasmic reticulum (ER), while the CTF (glycosylphosphatidylinositol [GPI] signal peptide [GPI-SP]) is cleaved by the GPI anchor. GPI anchor could be involved in PrP protein transport. It may also play a role of attachment of prion protein into the outer surface of cell membrane. Normal PrP is composed of a long N-terminal loop (which contains the octapeptide repeat region), two short β sheets, three α helices, and a C-terminal region (which contains the GPI anchor). Cleavage of PrP results in a 208 AA long glyocoprotein, anchored in the cell membrane.

[1080] The 253 amino acid sequence of PRNP (NP_000302.1) is as follows:

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYPPQGGG
GWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGTHSQWNKPSKPKT
NMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQV
YYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMCITQ
YERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 291).

[1081] The 253 amino acid sequence of PRNP (NP_000302.1) is encoded by the following nucleotide sequence (NCBI Ref. Seq No. NM_000311.5, "homo sapiens prion protein (PRNP), transcript variant 1, mRNA), is as follows:

GCGAACCTTGGCTGCTGGATGCTGGTTCTCTTTGTGGCCACATGGAGTGACCTGG
GCCTCTGCAAGAAGCGCCCGAAGCCTGGAGGATGGAACACTGGGGGCAGCCGAT
ACCCGGGGCAGGGCAGCCCTGGAGGCAACCGCTACCCACCTCAGGGCGGTGGTG
GCTGGGGGCAGCCTCATGGTGGTGGCTGGGGGCAGCCTCATGGTGGTGGCTGGG
GGCAGCCCCATGGTGGTGGCTGGGGACAGCCTCATGGTGGTGGCTGGGGTCAAG
GAGGTGGCACCCACAGTCAGTGGAACAAGCCGAGTAAGCCAAAAACCAACATGA
AGCACATGGCTGGTGCTGCAGCAGCTGGGGCAGTGGTGGGGGGCCTTGGCGGCT
ACATGCTGGGAAGTGCCATGAGCAGGCCCATCATACATTTCGGCAGTGACTATGAG
GACCGTTACTATCGTGAAAACATGCACCGTTACCCCAACCAAGTGTACTACAGGCC
CATGGATGAGTACAGCAACCAGAACAACTTTGTGCACGACTGCGTCAATATCACA
ATCAAGCAGCACACGGTCACCACAACCACCAAGGGGGAGAACTTCACCGAGACC
GACGTTAAGATGATGGAGCGCGTGGTTGAGCAGATGTGTATCACCCAGTACGAGA
GGGAATCTCAGGCCTATTACCAGAGAGGATCGAGCATGGTCCTCTTCTCCTCTCCA
CCTGTGATCCTCCTGATCTCTTTCCTCATCTTCCTGATAGTGGGATGAGGAAGGTCT
TCCTGTTTTCACCATCTTTCTAATCTTTTTCCAGCTTGAGGGAGGCGGTATCCACCT
GCAGCCCTTTTAGTGGTGGTGTCTCACTCTTTCTTCTCTCTTTGTCCCGGATAGGCT
AATCAATACCCTTGGCACTGATGGGCACTGGAAAACATAGAGTAGACCTGAGATG
CTGGTCAAGCCCCCTTTGATTGAGTTCATCATGAGCCGTTGCTAATGCCAGGCCAG
TAAAAGTATAACAGCAAATAACCATTGGTTAATCTGGACTTATTTTTGGACTTAGTG
CAACAGGTTGAGGCTAAAACAAATCTCAGAACAGTCTGAAATACCTTTGCCTGGA
TACCTCTGGCTCCTTCAGCAGCTAGAGCTCAGTATACTAATGCCCTATCTTAGTAGA
GATTTCATAGCTATTTAGAGATATTTTCCATTTTAAGAAAACCCGACAACATTTCTG
CCAGGTTTGTTAGGAGGCCACATGATACTTATTCAAAAAAATCCTAGAGATTCTTA
GCTCTTGGGATGCAGGCTCAGCCCGCTGGAGCATGAGCTCTGTGTGTACCGAGAA
CTGGGGTGATGTTTTACTTTTCACAGTATGGGCTACACAGCAGCTGTTCAACAAGA
GTAAATATTGTCACAACACTGAACCTCTGGCTAGAGGACATATTCACAGTGAACAT

AACTGTAACATATATGAAAGGCTTCTGGGACTTGAAATCAAATGTTTGGGAATGGT
GCCCTTGGAGGCAACCTCCCATTTTAGATGTTTAAAGGACCCTATATGTGGCATTCC
TTTCTTTAAACTATAGGTAATTAAGGCAGCTGAAAAGTAAATTGCCTTCTAGACAC
TGAAGGCAAATCTCCTTTGTCCATTTACCTGGAAACCAGAATGATTTTGACATACA
GGAGAGCTGCAGTTGTGAAAGCACCATCATCATAGAGGATGATGTAATTAAAAAAT
GGTCAGTGTGCAAAGAAAAGAACTGCTTGCATTTCTTTATTTCTGTCTCATAATTG
TCAAAAACCAGAATTAGGTCAAGTTCATAGTTTCTGTAATTGGCTTTTGAATCAAA
GAATAGGGAGACAATCTAAAAAATATCTTAGGTTGGAGATGACAGAAATATGATTG
ATTTGAAGTGGAAAAAGAAATTCTGTTAATGTTAATTAAAGTAAAATTATTCCCTG
AATTGTTTGATATTGTCACCTAGCAGATATGTATTACTTTTCTGCAATGTTATTATTG
GCTTGCACTTTGTGAGTATTCTATGTAAAAATATATATGTATATAAAATATATATTGCA
TAGGACAGACTTAGGAGTTTTGTTTAGAGCAGTTAACATCTGAAGTGTCTAATGCA
TTAACTTTTGTAAGGTACTGAATACTTAATATGTGGGAAACCCTTTTGCGTGGTCCT
TAGGCTTACAATGTGCACTGAATCGTTTCATGTAAGAATCCAAAGTGGACACCATT
AACAGGTCTTTGAAATATGCATGTACTTTATATTTTCTATATTTGTAACTTTGCATGT
TCTTGTTTTGTTATATAAAAAAATTGTAAATGTTTAATATCTGACTGAAATTAAACG
AGCGAAGATGAGCACCA (SEQ ID NO: 292)

[1082] Mutation sites relative to PRNP (NP_000302.1) which are linked to CJD and FFI are reported are as follows. These mutations can be removed or installed using the prime editors disclosed herein.

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO CJD PRION DISEASE (SEE TABLE 1 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)
D178N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHNCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 293)
T188K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHKVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 294)
E196K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGKNFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 295)
E196A	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT

	TTTKGANFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 296)
E200K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTKTDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 297)
E200G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTGTDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 298)
V203I	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDIKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 299)
R208H	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMEHVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 300)
V210I	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVIEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 301)
E211Q	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVQQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 302)
M232R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSRVLFSSPPV (SEQ ID NO: 303)

[1083] Mutation sites relative to PRNP (NP_000302.1) (SEQ ID NO: 291) which are linked to GSS are reported, as follows:

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO GSS PRION DISEASE (SEE TABLE 2 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)
P102L	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKLSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 304)
P105L	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKLKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 305)
A117V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAVAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 306)
G131V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLVSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 307)
V176G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFGHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 308)
H187R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQRTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 309)
	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 291)
F198S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT

	TTTKGENSTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 311)
D202N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETNVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 312)
Q212P	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEPMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 313)
Q217R	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITRYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 314)
M232T	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSTVLFSSPPV (SEQ ID NO: 315)

[1084] Mutation sites relative to PRNP (NP_000302.1) (SEQ ID NO: 291) which are linked to a possible protective nature against prion disease, as follows:

MUTATION	AMINO ACID SEQUENCE OF MUTANT PRNP LINKED TO A PROTECTIVE NATURE AGAINST PRION DISEASE (SEE TABLE 4 OF BAGYNSZKY ET AL., 2018) (RELATIVE TO SEQ ID NO: 291 OF PRNP NP_000302.1)
G127S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGSYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 316)
G127V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGVYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 317)
M129V	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYVLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT

	TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 318)
D167G	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMGEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 319)
D167N	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMNEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 320)
N171S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSSQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 321)
E219K	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYKRESQAYYQRGSSMVLFSSPPV (SEQ ID NO: 322)
P238S	MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNR YPPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGG GTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHF GSDYEDRYYRENMHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVT TTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQRGSSMVLFSSSPV (SEQ ID NO: 323)

[1085] Thus, in various embodiments, prime editing may be used to remove a mutation in PRNP that is linked to prion disease or install a mutation in PRNP that is considered to be protective against prion disease. For example, prime editing may be use to remove or restore a D178N, V180I, T188K, E196K, E196A, E200K, E200G, V203I, R208H, V210I, E211Q, I215V, or M232R mutation in the PRNP protein (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). In other embodiments, prime editing may be use to remove or restore a P102L, P105L, A117V, G131V, V176G, H187R, F198S, D202N, Q212P, Q217R, or M232T mutation in the PRNP protein (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). By removing or correcting for the presence of such mutations in PRNP using prime editing, the risk of prion disease may be reduced or eliminated.

[1086] In other embodiments, prime editing may be used to install a protective mutation in PRNP that is linked to a protective effect against one or more prion diseases. For example, prime editing may be used to install a G127S, G127V, M129V, D167G, D167N, N171S, E219K, or P238S protective mutation in PRNP (relative to PRNP of NP_000302.1) (SEQ ID NO: 291). In still other embodiments, the protective mutation may be any alternate amino acid installed at G127, G127, M129, D167, D167, N171, E219, or P238 in PRNP (relative to PRNP of NP_000302.1) (SEQ ID NO: 291).

[1087] In particular embodiments, prime editing may be used to install a G127V protective mutation in PRNP, as illustrated in FIG. 27 and discussed in Example 5.

[1088] In another embodiment, prime editing may be used to install an E219K protective mutation in PRNP.

[1089] The *PRNP* protein and the protective mutation site are conserved in mammals, so in addition to treating human disease it could also be used to generate cows and sheep that are immune to prion disease, or even help cure wild populations of animals that are suffering from prion disease. Prime editing has already been used to achieve ~25% installation of a naturally occurring protective allele in human cells, and previous mouse experiments indicate that this level of installation is sufficient to cause immunity from most prion diseases. This method is the first and potentially only current way to install this allele with such high efficiency in most cell types. Another possible strategy for treatment is to use prime editing to reduce or eliminate the expression of *PRNP* by installing an early stop codon in the gene.

[1090] Using the principles described herein for PEgRNA design, appropriate PEgRNAs may be designed for installing desired protective mutations, or for removing prion disease-associated mutations from PRNP. For example, the below list of PEgRNAs can be used to install the G127V protective allele and the E219K protective allele in human PRNP, as well as the G127V protective allele in PRNP of various animals.

[9] Pharmaceutical compositions

[1091] Other aspects of the present disclosure relate to pharmaceutical compositions comprising any of the various components of the prime editing system described herein (e.g., including, but not limited to, the napDNAbps, reverse transcriptases, fusion proteins (e.g., comprising napDNAbps and reverse transcriptases), extended guide RNAs, and complexes comprising fusion proteins and extended guide RNAs, as well as accessory elements, such as second strand nicking components and 5′ endogenous DNA flap removal endonucleases for helping to drive the prime editing process towards the edited product formation).

[1092] The term "pharmaceutical composition", as used herein, refers to a composition formulated for pharmaceutical use. In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises additional agents (e.g. for specific delivery, increasing half-life, or other therapeutic compounds).

[1093] As used here, the term "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the compound from one site (e.g., the delivery site) of the body, to another site (e.g., organ, tissue or portion of the body). A pharmaceutically acceptable carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the tissue of the subject (e.g., physiologically compatible, sterile, physiologic pH, etc.). Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C2-C12 alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

[1094] In some embodiments, the pharmaceutical composition is formulated for delivery to a subject, e.g., for gene editing. Suitable routes of administrating the pharmaceutical composition described herein include, without limitation: topical, subcutaneous, transdermal, intradermal, intralesional, intraarticular, intraperitoneal, intravesical, transmucosal, gingival, intradental, intracochlear, transtympanic, intraorgan, epidural, intrathecal, intramuscular, intravenous, intravascular, intraosseus, periocular, intratumoral, intracerebral, and intracerebroventricular administration.

[1095] In some embodiments, the pharmaceutical composition described herein is administered locally to a diseased site (e.g., tumor site). In some embodiments, the pharmaceutical composition described herein is administered to a subject by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including a membrane, such as a sialastic membrane, or a fiber.

[1096] In other embodiments, the pharmaceutical composition described herein is delivered in a controlled release system. In one embodiment, a pump may be used (see, e.g., Langer, 1990, Science 249:1527-1533; Sefton, 1989, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used. (See, e.g., Medical Applications of Controlled Release (Langer and Wise eds., CRC Press, Boca Raton, Fla., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., Wiley, New York, 1984); Ranger and Peppas, 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61. See also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105). Other controlled release systems are discussed, for example, in Langer, supra.

[1097] In some embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a composition adapted for intravenous or subcutaneous administration to a subject, e.g., a human. In some embodiments, pharmaceutical composition for administration by injection are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical can also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be

administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[1098] A pharmaceutical composition for systemic administration may be a liquid, e.g., sterile saline, lactated Ringer's or Hank's solution. In addition, the pharmaceutical composition can be in solid forms and re-dissolved or suspended immediately prior to use. Lyophilized forms are also contemplated.

[1099] The pharmaceutical composition can be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which is also suitable for parenteral administration. The particles can be of any suitable structure, such as unilamellar or plurilamellar, so long as compositions are contained therein. Compounds can be entrapped in "stabilized plasmid-lipid particles" (SPLP) containing the fusogenic lipid dioleoylphosphatidylethanolamine (DOPE), low levels (5-10 mol%) of cationic lipid, and stabilized by a polyethyleneglycol (PEG) coating (Zhang Y. P. *et al.*, *Gene Ther.* 1999, 6:1438-47). Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-amoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, *e.g.*, U.S. Patent Nos. 4,880,635; 4,906,477; 4,911,928; 4,917,951; 4,920,016; and 4,921,757; each of which is incorporated herein by reference.

[1100] The pharmaceutical composition described herein may be administered or packaged as a unit dose, for example. The term "unit dose" when used in reference to a pharmaceutical composition of the present disclosure refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

[1101] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a compound of the invention in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (e.g., sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the lyophilized compound of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[1102] In another aspect, an article of manufacture containing materials useful for the treatment of the diseases described above is included. In some embodiments, the article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container holds a composition that is effective for treating a disease described herein and may have a sterile access port. For example, the container may be an intravenous solution bag or a vial having a stopper pierce-able by a hypodermic injection needle. The active agent in the composition is a compound of the invention. In some embodiments, the label on or associated with the container indicates that the composition is used for treating the disease of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[10] Kits, cells, vectors, and delivery

Kits

[1103] The compositions of the present disclosure may be assembled into kits. In some embodiments, the kit comprises nucleic acid vectors for the expression of the prime editors described herein. In other embodiments, the kit further comprises appropriate guide nucleotide sequences (*e.g.*, PEgRNAs and second-site gRNAs) or nucleic acid vectors for the expression of such guide nucleotide sequences, to target the Cas9 protein or prime editor to the desired target sequence.

[1104] The kit described herein may include one or more containers housing components for performing the methods described herein and optionally instructions for use. Any of the kit described herein may further comprise components needed for performing the assay methods. Each component of the kits, where applicable, may be provided in liquid form (*e.g.*, in solution) or in solid form, (*e.g.*, a dry powder). In certain cases, some of the components may be reconstitutable or otherwise processible (*e.g.*, to an active form), for example, by the addition of a suitable solvent or other species (for example, water), which may or may not be provided with the kit.

[1105] In some embodiments, the kits may optionally include instructions and/or promotion for use of the components provided. As used herein, "instructions" can define a component of instruction and/or promotion, and typically involve written instructions on or associated with

packaging of the disclosure. Instructions also can include any oral or electronic instructions provided in any manner such that a user will clearly recognize that the instructions are to be associated with the kit, for example, audiovisual (*e.g.*, videotape, DVD, *etc.*), Internet, and/or web-based communications, *etc.* The written instructions may be in a form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological products, which can also reflect approval by the agency of manufacture, use or sale for animal administration. As used herein, "promoted" includes all methods of doing business including methods of education, hospital and other clinical instruction, scientific inquiry, drug discovery or development, academic research, pharmaceutical industry activity including pharmaceutical sales, and any advertising or other promotional activity including written, oral and electronic communication of any form, associated with the disclosure. Additionally, the kits may include other components depending on the specific application, as described herein.

[1106] The kits may contain any one or more of the components described herein in one or more containers. The components may be prepared sterilely, packaged in a syringe and shipped refrigerated. Alternatively it may be housed in a vial or other container for storage. A second container may have other components prepared sterilely. Alternatively the kits may include the active agents premixed and shipped in a vial, tube, or other container.

[1107] The kits may have a variety of forms, such as a blister pouch, a shrink wrapped pouch, a vacuum sealable pouch, a sealable thermoformed tray, or a similar pouch or tray form, with the accessories loosely packed within the pouch, one or more tubes, containers, a box or a bag. The kits may be sterilized after the accessories are added, thereby allowing the individual accessories in the container to be otherwise unwrapped. The kits can be sterilized using any appropriate sterilization techniques, such as radiation sterilization, heat sterilization, or other sterilization methods known in the art. The kits may also include other components, depending on the specific application, for example, containers, cell media, salts, buffers, reagents, syringes, needles, a fabric, such as gauze, for applying or removing a disinfecting agent, disposable gloves, a support for the agents prior to administration, *etc.* Some aspects of this disclosure provide kits comprising a nucleic acid construct comprising a nucleotide sequence encoding the various components of the prime editing system described herein (e.g., including, but not limited to, the napDNAbps, reverse transcriptases, polymerases, fusion proteins (e.g., comprising napDNAbps and reverse transcriptases (or more broadly, polymerases), extended guide RNAs, and complexes comprising fusion

proteins and extended guide RNAs, as well as accessory elements, such as second strand nicking components (e.g., second strand nicking gRNA) and 5′ endogenous DNA flap removal endonucleases for helping to drive the prime editing process towards the edited product formation). In some embodiments, the nucleotide sequence(s) comprises a heterologous promoter (or more than a single promoter) that drives expression of the prime editing system components.

[1108] Other aspects of this disclosure provide kits comprising one or more nucleic acid constructs encoding the various components of the prime editing system described herein, e.g., the comprising a nucleotide sequence encoding the components of the prime editing system capable of modifying a target DNA sequence. In some embodiments, the nucleotide sequence comprises a heterologous promoter that drives expression of the prime editing system components.

[1109] Some aspects of this disclosure provides kits comprising a nucleic acid construct, comprising (a) a nucleotide sequence encoding a napDNAbp (*e.g.,* a Cas9 domain) fused to a reverse transcriptase and (b) a heterologous promoter that drives expression of the sequence of (a).

Cells

[1110] Cells that may contain any of the compositions described herein include prokaryotic cells and eukaryotic cells. The methods described herein are used to deliver a Cas9 protein or a prime editor into a eukaryotic cell (*e.g.*, a mammalian cell, such as a human cell). In some embodiments, the cell is *in vitro* (*e.g.*, cultured cell. In some embodiments, the cell is *in vivo* (*e.g.*, in a subject such as a human subject). In some embodiments, the cell is *ex vivo* (*e.g.*, isolated from a subject and may be administered back to the same or a different subject).

[1111] Mammalian cells of the present disclosure include human cells, primate cells (*e.g.*, vero cells), rat cells (*e.g.*, GH3 cells, OC23 cells) or mouse cells (*e.g.*, MC3T3 cells). There are a variety of human cell lines, including, without limitation, human embryonic kidney (HEK) cells, HeLa cells, cancer cells from the National Cancer Institute's 60 cancer cell lines (NCI60), DU145 (prostate cancer) cells, Lncap (prostate cancer) cells, MCF-7 (breast cancer) cells, MDA-MB-438 (breast cancer) cells, PC3 (prostate cancer) cells, T47D (breast cancer) cells, THP-1 (acute myeloid leukemia) cells, U87 (glioblastoma) cells, SHSY5Y human neuroblastoma cells (cloned from a myeloma) and Saos-2 (bone cancer) cells. In some embodiments, rAAV vectors are delivered into human embryonic kidney (HEK) cells (*e.g.*, HEK 293 or HEK 293T cells). In some embodiments, rAAV vectors are delivered into stem

cells (*e.g.*, human stem cells) such as, for example, pluripotent stem cells (*e.g.*, human pluripotent stem cells including human induced pluripotent stem cells (hiPSCs)). A stem cell refers to a cell with the ability to divide for indefinite periods in culture and to give rise to specialized cells. A pluripotent stem cell refers to a type of stem cell that is capable of differentiating into all tissues of an organism, but not alone capable of sustaining full organismal development. A human induced pluripotent stem cell refers to a somatic (*e.g.*, mature or adult) cell that has been reprogrammed to an embryonic stem cell-like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells (*see*, *e.g.*, Takahashi and Yamanaka, *Cell* 126 (4): 663–76, 2006, incorporated by reference herein). Human induced pluripotent stem cell cells express stem cell markers and are capable of generating cells characteristic of all three germ layers (ectoderm, endoderm, mesoderm).

[1112] Additional non-limiting examples of cell lines that may be used in accordance with the present disclosure include 293-T, 293-T, 3T3, 4T1, 721, 9L, A-549, A172, A20, A253, A2780, A2780ADR, A2780cis, A431, ALC, B16, B35, BCP-1, BEAS-2B, bEnd.3, BHK-21, BR 293, BxPC3, C2C12, C3H-10T1/2, C6, C6/36, Cal-27, CGR8, CHO, CML T1, CMT, COR-L23, COR-L23/5010, COR-L23/CPR, COR-L23/R23, COS-7, COV-434, CT26, D17, DH82, DU145, DuCaP, E14Tg2a, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, Hepa1c1c7, High Five cells, HL-60, HMEC, HT-29, HUVEC, J558L cells, Jurkat, JY cells, K562 cells, KCL22, KG1, Ku812, KYO1, LNCap, Ma-Mel 1, 2, 3....48, MC-38, MCF-10A, MCF-7, MDA-MB-231, MDA-MB-435, MDA-MB-468, MDCK II, MG63, MONO-MAC 6, MOR/0.2R, MRC5, MTD-1A, MyEnd, NALM-1, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NW-145, OPCN/OPCT Peer, PNT-1A/PNT 2, PTK2, Raji, RBL cells, RenCa, RIN-5F, RMA/RMAS, S2, Saos-2 cells, Sf21, Sf9, SiHa, SKBR3, SKOV-3, T-47D, T2, T84, THP1, U373, U87, U937, VCaP, WM39, WT-49, X63, YAC-1 and YAR cells.

[1113] Some aspects of this disclosure provide cells comprising any of the constructs disclosed herein. In some embodiments, a host cell is transiently or non-transiently transfected with one or more vectors described herein. In some embodiments, a cell is transfected as it naturally occurs in a subject. In some embodiments, a cell that is transfected is taken from a subject. In some embodiments, the cell is derived from cells taken from a subject, such as a cell line. A wide variety of cell lines for tissue culture are known in the art. Examples of cell lines include, but are not limited to, C8161, CCRF-CEM, MOLT, mIMCD-

3, NHDF, HeLa-S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEKn, HEKa, MiaPaCell, Panc1, PC-3, TF1, CTLL-2, C1R, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu1, SW480, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, TIB55, Jurkat, J45.01, LRMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, 132-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, A2780ADR, A2780cis, A 172, A20, A253, A431, A-549, ALC, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293. BxPC3. C3H-10T1/2, C6/36, Cal-27, CHO, CHO-7, CHO-IR, CHO-K1, CHO-K2, CHO-T, CHO Dhfr –/–, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV-434, CML T1, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, HEK-293, HeLa, Hepa1c1c7, HL-60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KYO1, LNCap, Ma-Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK 11, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NALM-1, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-5F, RMA/RMAS, Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-1, YAR, and transgenic varieties thereof.

[1114] Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC) (Manassus, Va.)). In some embodiments, a cell transfected with one or more vectors described herein is used to establish a new cell line comprising one or more vector-derived sequences. In some embodiments, a cell transiently transfected with the components of a CRISPR system as described herein (such as by transient transfection of one or more vectors, or transfection with RNA), and modified through the activity of a CRISPR complex, is used to establish a new cell line comprising cells containing the modification but lacking any other exogenous sequence. In some embodiments, cells transiently or non-transiently transfected with one or more vectors described herein, or cell lines derived from such cells are used in assessing one or more test compounds.

Vectors

[1115] Some aspects of the present disclosure relate to using recombinant virus vectors (e.g., adeno-associated virus vectors, adenovirus vectors, or herpes simplex virus vectors) for the

delivery of the prime editors or components thereof described herein, e.g., the split Cas9 protein or a split nucleobase prime editors, into a cell. In the case of a split-PE approach, the N-terminal portion of a PE fusion protein and the C-terminal portion of a PE fusion are delivered by separate recombinant virus vectors (e.g., adeno-associated virus vectors, adenovirus vectors, or herpes simplex virus vectors) into the same cell, since the full-length Cas9 protein or prime editors exceeds the packaging limit of various virus vectors, e.g., rAAV (~4.9 kb).

[1116] Thus, in one embodiment, the dislosure contemplates vectors capable of delivering split prime editor fusion proteins, or split components thereof. In some embodiments, a composition for delivering the split Cas9 protein or split prime editor into a cell (*e.g.*, a mammalian cell, a human cell) is provided. In some embodiments, the composition of the present disclosure comprises: (i) a first recombinant adeno-associated virus (rAAV) particle comprising a first nucleotide sequence encoding a N-terminal portion of a Cas9 protein or prime editor fused at its C-terminus to an intein-N; and (ii) a second recombinant adeno-associated virus (rAAV) particle comprising a second nucleotide sequence encoding an intein-C fused to the N-terminus of a C-terminal portion of the Cas9 protein or prime editor. The rAAV particles of the present disclosure comprise a rAAV vector (*i.e.*, a recombinant genome of the rAAV) encapsidated in the viral capsid proteins.

[1117] In some embodiments, the rAAV vector comprises: (1) a heterologous nucleic acid region comprising the first or second nucleotide sequence encoding the N-terminal portion or C-terminal portion of a split Cas9 protein or a split prime editor in any form as described herein, (2) one or more nucleotide sequences comprising a sequence that facilitates expression of the heterologous nucleic acid region (*e.g.*, a promoter), and (3) one or more nucleic acid regions comprising a sequence that facilitate integration of the heterologous nucleic acid region (optionally with the one or more nucleic acid regions comprising a sequence that facilitates expression) into the genome of a cell. In some embodiments, viral sequences that facilitate integration comprise Inverted Terminal Repeat (ITR) sequences. In some embodiments, the first or second nucleotide sequence encoding the N-terminal portion or C-terminal portion of a split Cas9 protein or a split prime editor is flanked on each side by an ITR sequence. In some embodiments, the nucleic acid vector further comprises a region encoding an AAV Rep protein as described herein, either contained within the region flanked by ITRs or outside the region. The ITR sequences can be derived from any AAV serotype

(*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) or can be derived from more than one serotype. In some embodiments, the ITR sequences are derived from AAV2 or AAV6.

[1118] Thus, in some embodiments, the rAAV particles disclosed herein comprise at least one rAAV2 particle, rAAV6 particle, rAAV8 particle, rPHP.B particle, rPHP.eB particle, or rAAV9 particle, or a variant thereof. In particular embodiments, the disclosed rAAV particles are rPHP.B particles, rPHP.eB particles, rAAV9 particles.

[1119] ITR sequences and plasmids containing ITR sequences are known in the art and commercially available (see, *e.g.*, products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; and Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Proc Natl Acad Sci USA. 1996 Nov 26;93(24):14082-7; and Curtis A. Machida. Methods in Molecular Medicine™. Viral Vectors for Gene Therapy Methods and Protocols. 10.1385/1-59259-304-6:201 © Humana Press Inc. 2003. Chapter 10. Targeted Integration by Adeno-Associated Virus. Matthew D. Weitzman, Samuel M. Young Jr., Toni Cathomen and Richard Jude Samulski; U.S. Pat. Nos. 5,139,941 and 5,962,313, all of which are incorporated herein by reference).

[1120] In some embodiments, the rAAV vector of the present disclosure comprises one or more regulatory elements to control the expression of the heterologous nucleic acid region (*e.g.*, promoters, transcriptional terminators, and/or other regulatory elements). In some embodiments, the first and/or second nucleotide sequence is operably linked to one or more (*e.g.*, 1, 2, 3, 4, 5, or more) transcriptional terminators. Non-limiting examples of transcriptional terminators that may be used in accordance with the present disclosure include transcription terminators of the bovine growth hormone gene (bGH), human growth hormone gene (hGH), SV40, CW3, ϕ, or combinations thereof. The efficiencies of several transcriptional terminators have been tested to determine their respective effects in the expression level of the split Cas9 protein or the split prime editor. In some embodiments, the transcriptional terminator used in the present disclosure is a bGH transcriptional terminator. In some embodiments, the rAAV vector further comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE). In certain embodiments, the WPRE is a truncated WPRE sequence, such as "W3." In some embodiments, the WPRE is inserted 5′ of

the transcriptional terminator. Such sequences, when transcribed, create a tertiary structure which enhances expression, in particular, from viral vectors.

[1121] In some embodiments, the vectors used herein may encode the PE fusion proteins, or any of the components thereof (e.g., napDNAbp, linkers, or polymerases). In addition, the vectors used herein may encode the PEgRNAs, and/or the accessory gRNA for second strand nicking. The vectors may be capable of driving expression of one or more coding sequences in a cell. In some embodiments, the cell may be a prokaryotic cell, such as, e.g., a bacterial cell. In some embodiments, the cell may be a eukaryotic cell, such as, e.g., a yeast, plant, insect, or mammalian cell. In some embodiments, the eukaryotic cell may be a mammalian cell. In some embodiments, the eukaryotic cell may be a rodent cell. In some embodiments, the eukaryotic cell may be a human cell. Suitable promoters to drive expression in different types of cells are known in the art. In some embodiments, the promoter may be wild-type. In other embodiments, the promoter may be modified for more efficient or efficacious expression. In yet other embodiments, the promoter may be truncated yet retain its function. For example, the promoter may have a normal size or a reduced size that is suitable for proper packaging of the vector into a virus.

[1122] In some embodiments, the promoters that may be used in the prime editor vectors may be constitutive, inducible, or tissue-specific. In some embodiments, the promoters may be a constitutive promoters. Non-limiting exemplary constitutive promoters include cytomegalovirus immediate early promoter (CMV), simian virus (SV40) promoter, adenovirus major late (MLP) promoter, Rous sarcoma virus (RSV) promoter, mouse mammary tumor virus (MMTV) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-alpha (EFla) promoter, ubiquitin promoters, actin promoters, tubulin promoters, immunoglobulin promoters, a functional fragment thereof, or a combination of any of the foregoing. In some embodiments, the promoter may be a CMV promoter. In some embodiments, the promoter may be a truncated CMV promoter. In other embodiments, the promoter may be an EFla promoter. In some embodiments, the promoter may be an inducible promoter. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech). In some embodiments, the promoter may be a tissue-specific promoter. In some embodiments, the tissue-specific promoter is exclusively or predominantly expressed in liver tissue. Non-limiting exemplary

tissue-specific promoters include B29 promoter, CD14 promoter, CD43 promoter, CD45 promoter, CD68 promoter, desmin promoter, elastase- 1 promoter, endoglin promoter, fibronectin promoter, Flt-1 promoter, GFAP promoter, GPIIb promoter, ICAM- 2 promoter, INF-β promoter, Mb promoter, Nphsl promoter, OG-2 promoter, SP-B promoter, SYN1 promoter, and WASP promoter.

[1123] In some embodiments, the prime editor vectors (e.g., including any vectors encoding the prime editor fusion protein and/or the PEgRNAs, and/or the accessory second strand nicking gRNAs) may comprise inducible promoters to start expression only after it is delivered to a target cell. Non-limiting exemplary inducible promoters include those inducible by heat shock, light, chemicals, peptides, metals, steroids, antibiotics, or alcohol. In some embodiments, the inducible promoter may be one that has a low basal (non-induced) expression level, such as, e.g., the Tet-On® promoter (Clontech).

[1124] In additional embodiments, the prime editor vectors (e.g., including any vectors encoding the prime editor fusion protein and/or the PEgRNAs, and/or the accessory second strand nicking gRNAs) may comprise tissue- specific promoters to start expression only after it is delivered into a specific tissue. Non-limiting exemplary tissue-specific promoters include B29 promoter, CD14 promoter, CD43 promoter, CD45 promoter, CD68 promoter, desmin promoter, elastase- 1 promoter, endoglin promoter, fibronectin promoter, Flt-1 promoter, GFAP promoter, GPIIb promoter, ICAM- 2 promoter, INF-β promoter, Mb promoter, Nphsl promoter, OG-2 promoter, SP-B promoter, SYN1 promoter, and WASP promoter.

[1125] In some embodiments, the nucleotide sequence encoding the PEgRNA (or any guide RNAs used in connection with prime editing) may be operably linked to at least one transcriptional or translational control sequence. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to at least one promoter. In some embodiments, the promoter may be recognized by RNA polymerase III (Pol III). Non-limiting examples of Pol III promoters include U6, HI and tRNA promoters. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human U6 promoter. In other embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human HI promoter. In some embodiments, the nucleotide sequence encoding the guide RNA may be operably linked to a mouse or human tRNA promoter. In embodiments with more than one guide RNA, the promoters used to drive expression may be the same or different. In some embodiments, the nucleotide encoding the crRNA of the guide RNA and the nucleotide encoding the tracr RNA

of the guide RNA may be provided on the same vector. In some embodiments, the nucleotide encoding the crRNA and the nucleotide encoding the tracr RNA may be driven by the same promoter. In some embodiments, the crRNA and tracr RNA may be transcribed into a single transcript. For example, the crRNA and tracr RNA may be processed from the single transcript to form a double-molecule guide RNA. Alternatively, the crRNA and tracr RNA may be transcribed into a single-molecule guide RNA.

[1126] In some embodiments, the nucleotide sequence encoding the guide RNA may be located on the same vector comprising the nucleotide sequence encoding the PE fusion protein. In some embodiments, expression of the guide RNA and of the PE fusion protein may be driven by their corresponding promoters. In some embodiments, expression of the guide RNA may be driven by the same promoter that drives expression of the PE fusion protein. In some embodiments, the guide RNA and the PE fusion protein transcript may be contained within a single transcript. For example, the guide RNA may be within an untranslated region (UTR) of the Cas9 protein transcript. In some embodiments, the guide RNA may be within the 5' UTR of the PE fusion protein transcript. In other embodiments, the guide RNA may be within the 3' UTR of the PE fusion protein transcript. In some embodiments, the intracellular half-life of the PE fusion protein transcript may be reduced by containing the guide RNA within its 3' UTR and thereby shortening the length of its 3' UTR. In additional embodiments, the guide RNA may be within an intron of the PE fusion protein transcript. In some embodiments, suitable splice sites may be added at the intron within which the guide RNA is located such that the guide RNA is properly spliced out of the transcript. In some embodiments, expression of the Cas9 protein and the guide RNA in close proximity on the same vector may facilitate more efficient formation of the CRISPR complex.

[1127] The prime editor vector system may comprise one vector, or two vectors, or three vectors, or four vectors, or five vector, or more. In some embodiments, the vector system may comprise one single vector, which encodes both the PE fusion protein and PEgRNA. In other embodiments, the vector system may comprise two vectors, wherein one vector encodes the PE fusion protein and the other encodes the PEgRNA. In additional embodiments, the vector system may comprise three vectors, wherein the third vector encodes the second strand nicking gRNA used in the herein methods.

[1128] In some embodiments, the composition comprising the rAAV particle (in any form contemplated herein) further comprises a pharmaceutically acceptable carrier. In some

embodiments, the composition is formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects.

[1129] Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C2-C12 alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

Delivery methods

[1130] In some aspects, the invention provides methods comprising delivering one or more polynucleotides, such as or one or more vectors as described herein, one or more transcripts thereof, and/or one or proteins transcribed therefrom, to a host cell. In some aspects, the invention further provides cells produced by such methods, and organisms (such as animals, plants, or fungi) comprising or produced from such cells. In some embodiments, a base editor as described herein in combination with (and optionally complexed with) a guide sequence is delivered to a cell.

[1131] Exemplary delivery strategies are described herein elsewhere, which include vector-based strategies, PE ribonucleoprotein complex delivery, and delivery of PE by mRNA methods.

[1132] In some embodiments, the method of delivery provided comprises nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA.

[1133] Exemplary methods of delivery of nucleic acids include lipofection, nucleofection, electoporation, stable genome integration (e.g., piggybac), microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™, Lipofectin™ and SF Cell Line 4D-Nucleofector X Kit™ (Lonza)). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424; WO 91/16024. Delivery may be to cells (e.g. *in vitro* or *ex vivo* administration) or target tissues (e.g. *in vivo* administration). Delivery may be achieved through the use of RNP complexes.

[1134] The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese *et al., Cancer Gene Ther.* 2:291-297 (1995); Behr *et al., Bioconjugate Chem.* 5:382-389 (1994); Remy *et al., Bioconjugate Chem.* 5:647-654 (1994); Gao *et al., Gene Therapy* 2:710-722 (1995); Ahmad *et al., Cancer Res.* 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[1135] In other embodiments, the method of delivery and vector provided herein is an RNP complex. RNP delivery of fusion proteins markedly increases the DNA specificity of base editing. RNP delivery of fusion proteins leads to decoupling of on- and off-target DNA editing. RNP delivery ablates off-target editing at non-repetitive sites while maintaining on-target editing comparable to plasmid delivery, and greatly reduces off-target DNA editing even at the highly repetitive VEGFA site 2. See Rees, H.A. *et al.,* Improving the DNA specificity and applicability of base editing through protein engineering and protein delivery, *Nat. Commun.* 8, 15790 (2017), U.S. Patent No. 9,526,784, issued December 27, 2016, and U.S. Patent No. 9,737,604, issued August 22, 2017, each of which is incorporated by reference herein.

[1136] Additional methods for the delivery of nucleic acids to cells are known to those skilled in the art. See, for example, US 2003/0087817, incorporated herein by reference.

[1137] Other aspects of the present disclosure provide methods of delivering the prime editor constructs into a cell to form a complete and functional prime editor within a cell. For example, in some embodiments, a cell is contacted with a composition described herein (*e.g.*, compositions comprising nucleotide sequences encoding the split Cas9 or the split prime editor or AAV particles containing nucleic acid vectors comprising such nucleotide sequences). In some embodiments, the contacting results in the delivery of such nucleotide sequences into a cell, wherein the N-terminal portion of the Cas9 protein or the prime editor and the C-terminal portion of the Cas9 protein or the prime editor are expressed in the cell and are joined to form a complete Cas9 protein or a complete prime editor.

[1138] It should be appreciated that any rAAV particle, nucleic acid molecule or composition provided herein may be introduced into the cell in any suitable way, either stably or transiently. In some embodiments, the disclosed proteins may be transfected into the cell. In some embodiments, the cell may be transduced or transfected with a nucleic acid molecule. For example, a cell may be transduced (e.g., with a virus encoding a split protein), or transfected (e.g., with a plasmid encoding a split protein) with a nucleic acid molecule that encodes a split protein, or an rAAV particle containing a viral genome encoding one or more nucleic acid molecules. Such transduction may be a stable or transient transduction. In some embodiments, cells expressing a split protein or containing a split protein may be transduced or transfected with one or more guide RNA sequences, for example in delivery of a split Cas9 (e.g., nCas9) protein. In some embodiments, a plasmid expressing a split protein may be introduced into cells through electroporation, transient (e.g., lipofection) and stable genome integration (e.g., piggybac) and viral transduction or other methods known to those of skill in the art.

[1139] In certain embodiments, the compositions provided herein comprise a lipid and/or polymer. In certain embodiments, the lipid and/or polymer is cationic. The preparation of such lipid particles is well known. See, e.g. U.S. Patent Nos. 4,880,635; 4,906,477; 4,911,928; 4,917,951; 4,920,016; 4,921,757; and 9,737,604, each of which is incorporated herein by reference.

[1140] The guide RNA sequence may be 15-100 nucleotides in length and comprise a sequence of at least 10, at least 15, or at least 20 contiguous nucleotides that is complementary to a target nucleotide sequence. The guide RNA may comprise a sequence of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that is complementary to a target nucleotide sequence. The

guide RNA may be 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length.

[1141] In some embodiments, the target nucleotide sequence is a DNA sequence in a genome, e.g. a eukaryotic genome. In certain embodiments, the target nucleotide sequence is in a mammalian (e.g. a human) genome.

[1142] The compositions of this disclosure may be administered or packaged as a unit dose, for example. The term "unit dose" when used in reference to a pharmaceutical composition of the present disclosure refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent, *i.e.*, a carrier or vehicle.

[1143] Treatment of a disease or disorder includes delaying the development or progression of the disease, or reducing disease severity. Treating the disease does not necessarily require curative results.

[1144] As used therein, "delaying" the development of a disease means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. A method that "delays" or alleviates the development of a disease, or delays the onset of the disease, is a method that reduces probability of developing one or more symptoms of the disease in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a number of subjects sufficient to give a statistically significant result.

[1145] "Development" or "progression" of a disease means initial manifestations and/or ensuing progression of the disease. Development of the disease can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset.

[1146] As used herein "onset" or "occurrence" of a disease includes initial onset and/or recurrence. Conventional methods, known to those of ordinary skill in the art of medicine, can be used to administer the isolated polypeptide or pharmaceutical composition to the subject, depending upon the type of disease to be treated or the site of the disease.

[1147] Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

EXAMPLE 1. PRIME EDITING (PE) FOR INSTALLING PRECISE NUCLEOTIDE CHANGES IN THE GENOME

[1148] The objective is to develop a transformative genome editing technology for precise and general installation of single nucleotide changes in mammalian genomes. This technology would allow investigators to study the effects of single nucleotide variations in virtually any mammalian gene, and potentially enable therapeutic interventions for correcting pathogenic point mutations in human patients.

[1149] Adoption of the clustered regularly interspaced short palindromic repeat (CRISPR) system for genome editing has revolutionized the life sciences[1–3]. Although gene disruption using CRISPR is now routine, the precise installation of single nucleotide edits remains a major challenge, despite being necessary for studying or correcting a large number of disease-causative mutations. Homology directed repair (HDR) is capable of achieving such edits, but suffers from low efficiency (often <5%), a requirement for donor DNA repair templates, and deleterious effects of double-stranded DNA break (DSB) formation. Recently, the Liu laboratory developed base editing, which achieves efficient single nucleotide editing without DSBs. Base editors (BEs) combine the CRISPR system with base-modifying deaminase enzymes to convert target C•G or A•T base pairs to A•T or G•C, respectively[4–6]. Although already widely used by researchers worldwide (> 5,000 Liu lab BE constructs distributed by Addgene), current BEs enable only four of the twelve possible base pair conversions and are unable to correct small insertions or deletions. Moreover, the targeting scope of base editing is limited by the editing of non-target C or A bases adjacent to the target base ("bystander editing") and by the requirement that a PAM sequence exist 15±2 bp from the target base. Overcoming these limitations would therefore greatly broaden the basic research and therapeutic applications of genome editing.

[1150] Here, it is proposed to develop a new precision editing approach that offers many of the benefits of base editing—namely, avoidance of double strand breaks and donor DNA

repair templates—while overcoming its major limitations. To achieve this ambitious goal, it is aimed to directly install edited DNA strands at target genomic sites using target-primed reverse transcription (TPRT). In the design discussed herein, CRISPR guide RNA (gRNA) will be engineered to carry a template encoding mutagenic DNA strand synthesis, to be executed by an associated reverse transcriptase (RT) enzyme. The CRISPR nuclease (Cas9)-nicked target site DNA will serve as the primer for reverse transcription, allowing for direct incorporation of any desired nucleotide edit.

Section 1

[1151] Establish guide RNA-templated reverse transcription of mutagenic DNA strands. Prior studies have shown that, following DNA cleavage but prior to complex dissociation, Cas9 releases the non-target DNA strand to expose a free 3′ terminus. It is hypothesized that this DNA strand is accessible to extension by polymerase enzymes, and that gRNAs can be engineered through extension of their 5′ or 3′ terminus to serve as templates for DNA synthesis. In preliminary *in vitro* studies, it was established that nicked DNA strands within Cas9:gRNA-bound complexes can indeed prime reverse transcription using the bound gRNA as a template (RT enzyme in trans). Next, different gRNA linkers, primer binding sites, and synthesis templates will be explored to determine optimal design rules *in vitro*. Then, different RT enzymes, acting in trans or as fusions to Cas9, will be evaluated *in vitro*. Finally, engineered gRNA designs will be identified that retain efficient binding and cutting activity in cells. Successful demonstration of this aim will provide a foundation for carrying out mutagenic strand synthesis in cells.

Section 2

[1152] Establish prime editing in human cells. Based on DNA processing and repair mechanisms, it is hypothesized that mutagenic DNA strands (single stranded flaps) can be used to direct specific and efficient editing of target nucleotides. In encouraging preliminary studies, feasibility for this strategy was established by demonstrating editing with model plasmid substrates containing mutagenic flaps. Concurrent with Aim 1, repair outcomes will be further evaluated by systematically varying the mutagenic flap's length, sequence composition, target nucleotide identity, and 3′ terminus. Small 1 to 3 nucleotide insertions and deletions will also be tested. In parallel, and building from Aim 1, Cas9-RT architectures will be evaluated, including fusion proteins and non-covalent recruitment strategies. Cas9-RT architectures and extended gRNAs will be assayed for cellular editing at multiple target sites in the human genome, and will then be optimized for high efficiency. If successful, this

aim would immediately establish TPRT genome editing (i.e., prime editing) for basic science applications.

Section 3

[1153] Achieve site-specific editing of pathogenic mutations in cultured human cells. The potential generality of this technology could enable editing of transversion mutations and indels that are not currently correctable by BEs. Guided by the results of Aim 1 and Aim 2, pathogenic transversion mutations will be targeted in cultured human cells, including the sickle cell disease founder mutation in beta globin (requires an A•T to T•A transversion to correct) and the most prevalent Wilson's disease mutation in ATP7B (requires a G•C to T•A transversion to correct). The correction of small insertion and deletion mutations will also be examined, including the 3-nucleotide ΔF508 deletion in *CFTR* that causes cystic fibrosis. If successful, this would lay the foundation for developing powerful therapeutic approaches that address these important human diseases.

Approach

[1154] The objective is to develop a genome editing strategy that directly installs point mutations at targeted genomic sites. In the technology development phase, efforts will focus on protein and RNA engineering to incorporate TPRT functionality into the CRISPR/Cas system. *In vitro* assays will be used to carefully probe the function of each step of TPRT, building from the ground up (Aim 1). The second focus area will evaluate editing outcomes in mammalian cells using a combination of model substrates and engineered CRISPR/Cas systems (Aim 2). Finally, the application phase will use the technology to correct mutations that have been intractable to genome editing by other methods (Aim 3).

[1155] The general editing design is shown in FIGs. 1A-1B. Cas9 nickases contain inactivating mutations to the HNH nuclease domain (Spy Cas9 H840A or N863A), restricting DNA cleavage to the PAM containing strand (non-target strand). Guide RNAs (gRNAs) are engineered to contain a template for reverse transcription (designs detailed on slide 5). Shown is a 5′ extension of the gRNA, but 3′ extensions can also be implemented. The Cas9 nickase is fused to a reverse transcriptase (RT) enzyme, either through the C-terminus or N-terminus. The gRNA:Cas9-RT complex targets the DNA region of interest and forms an R loop after displacing the non-target strand. Cas9 nicks the non-target DNA strand. Release of the nicked strand exposes a free 3′-OH terminus that is competent to prime reverse transcription using the extended gRNA as a template. This DNA synthesis reaction is carried out by the fused RT enzyme. The gRNA template encodes a DNA sequence that is homologous to the original

DNA duplex, with the exception of the nucleotide that is targeted for editing. The product of reverse transcription is a single stranded DNA flap that encodes the desired edit. This flap, which contains a free 3′ terminus, can equilibrate with the adjacent DNA strand, resulting in a 5′ flap species. The latter species is hypothesized to serve as an efficient substrate for FEN1 (flap endonuclease 1), an enzyme that naturally excises 5′ flaps from Okazaki fragments during lagging strand DNA synthesis, and removes 5′ flaps following strand displacement synthesis that occurs during long-patch base excision repair. Ligation of the nicked DNA produces a mismatched base pair. This intermediate could either undergo reversion to the original base pair or conversion to the desired edited base pair via mismatch repair (MMR) processes. Alternatively, semiconservative DNA replication could give rise to one copy each of the reversion and edit.

1. Establish guide RNA-templated reverse transcription of mutagenic DNA strands.

Background and rationale

[1156] In the proposed genome editing strategy, the Cas9-nicked non-target DNA strand (PAM-containing strand that forms the R-loop) acts as the primer for DNA synthesis. It is hypothesized that this is possible based on several pieces of biochemical and structural data. Nuclease protection experiments[32], crystallographic studies[33], and base editing windows[4,24] have demonstrated a large degree of flexibility and disorder for the non-target strand nucleotides -20 through -10 within the so-called R-loop of the Cas9-bound complex (numbering indicates distance 5′ from first PAM nucleotide). Moreover, the PAM-distal portion of the cleaved non-target strand can be displaced from tightly bound ternary complexes when complementary ssDNA is added in trans[20]. These studies support that the non-target strand is highly flexible, is accessible to enzymes, and that after nicking, the 3′ terminus of the PAM-distal fragment is released prior to Cas9 dissociation. Furthermore, it is hypothesized that gRNAs can be extended to template DNA synthesis. Prior studies have shown that gRNAs for SpCas9, SaCas9, and LbCas12a (formerly Cpf1) tolerate gRNA extensions with RNA aptamers[34], ligand-inducible self-cleaving ribozymes[35], and long non-coding RNAs[36]. This literature establishes precedent for two major features that will be exploited. In assessing this strategy, several CRISPR-Cas systems will be evaluated in conjunction with 5′ and 3′ extended gRNA designs using a combination of *in vitro* and cellular assays (FIGs. 2A-2C).

[1157] Designs for engineered gRNAs for TRT editing are shown in FIGs. 3A-3B. DNA synthesis proceeds 5′ to 3′, and thus copies the RNA template in the 3′ to 5′ direction. The

design for the 5′ extension contains a linker region, a primer binding site where the nicked DNA strand anneals, and a template for DNA synthesis by reverse transcription. The 3′ extended gRNA contains a primer binding site and a reverse transcription template. In some cases, the 3′ RNA hairpin of the gRNA core is modified to match the DNA target sequence, as *in vitro* experiments showed that reverse transcription extends ~3 nucleotides into the gRNA core for the 3′ extended gRNA constructs (modification of the hairpin sequence appears well tolerated so long as compensatory changes are made that maintain the hairpin RNA structure). DNA synthesis proceed 5′ to 3′, with nucleotides added to the 3′ OH of the growing DNA strand.

Preliminary results

[1158] Cas9 nicked DNA primes reverse transcription of gRNA templates. To evaluate the accessibility of the nicked non-target DNA strand, *in vitro* biochemical assays were performed using the Cas9 nuclease from *S. pyogenes* (SpCas9) and Cy5 fluorescently labeled duplex DNA substrates (51 base pairs). First, a series of gRNAs containing 5′ extensions with varying synthesis template lengths were prepared by *in vitro* transcription (overall design shown in FIG. 2B). Electrophoretic mobility shift assays (EMSA) with nuclease dead Cas9 (dCas9) established that 5′ extended gRNAs maintain target binding affinity (data not shown). Next, TPRT activity was tested on pre-nicked Cy5-labeled duplex DNA substrates using dCas9, 5′-extended gRNAs, and Molony-Murine Leukemia Virus (M-MLV) reverse transcriptase (Superscript III). After 1 hour of incubation at 37 °C, products were evaluated by denaturing polyacrylamide gel electrophoresis (PAGE) and imaged using Cy5 fluorescence (FIG. 4A). Each 5′-extended gRNA variant led to significant product formation, with the observed DNA product sizes being consistent with the length of the extension template (FIG. 4B). Importantly, in the absence of dCas9, pre-nicked substrates were extended to the full 51-bp length of the DNA substrate, strongly suggesting that the complementary DNA strand, and not the gRNA, was used as the template for DNA synthesis when dCas9 was not present (FIG. 4C). Of note, the system was designed such that the newly synthesized DNA strand mirrors the product that would be required for target site editing (a homologous strand with a single nucleotide change). This result establishes that Cas9:gRNA binding exposes the nicked non-target strand's 3′ end, and that the non-target strand is accessible to reverse transcription.

[1159] Next, non-nicked dsDNA substrates were evaluated using the Cas9(H840A) mutant, which nicks the non-target DNA strand. First, to test Cas9(H840A) nickase activity with 5′-

extended gRNAs, *in vitro* cleavage assays were performed as previously described[37]. Although nicking was impaired by comparison to the standard gRNA, appreciable cleavage products were formed (FIG. 4D). Importantly, RT products were also observed when TPRT reactions were carried out with 5′-extended gRNAs and Cas9(H840A), albeit at lower yields that are likely explained by the decreased nicking activity (FIG. 4D). This result establishes that 5′-extended gRNA:Cas9(H840A) complexes can nick DNA and template reverse transcription.

[1160] Finally, 3′ gRNA extensions were evaluated for Cas9(H840A) nicking and TPRT. By comparison to 5′-extended gRNAs, DNA cleavage by 3′-extended gRNAs was not impaired to any detectable extent compared to the standard gRNA. Significantly, 3′-extended gRNA templates also supported efficient reverse transcription with both pre-nicked and intact duplex DNA substrates when M-MLV RT was supplied in trans (FIG. 4E). Surprisingly, only a single product was observed for 3′-extended templates, indicating that reverse transcription terminates at a specific location along the gRNA scaffold. Homopolymer tailing of the product with terminal transferase followed by Klenow extension and Sanger sequencing revealed that the full gRNA synthesis template was copied in addition to the terminal 3 nucleotides of the gRNA core. In the future, the flap terminus will be reprogrammed by modifying the terminal gRNA sequence[38,39]. This result demonstrates that 3′-extended gRNAs can serve as efficient nuclease targeting guides and can template reverse transcription.

[1161] Cas9-TPRT uses nicked DNA and gRNA in cis. Dual color experiments were used to determine if the RT reaction preferentially occurs with the gRNA in cis (bound in the same complex) (see FIG. 8). Two separate experiments were conducted for 5′-extended and 3′-extended gRNAs. For a given experiment, ternary complexes of dCas9, gRNA, and DNA substrate were formed in separate tubes. In one tube, the gRNA encodes a long RT product and the DNA substrate is labeled with Cy3 (red); in the other, the gRNA encodes a short RT product and the DNA substrate is labeled with Cy5 (blue). After short incubations, the complexes were mixed and then treated with RT enzyme and dNTPs. Products were separated by urea-denaturing PAGE and visualized by fluorescence in the Cy3 and Cy5 channels. Reaction products were found to preferentially form using the gRNA template that was pre-complexed with the DNA substrate, indicating that the RT reaction likely can occur in cis. This results supports that a single Cas9:gRNA complex can target a DNA site and template reverse transcription of a mutagenic DNA strand.

Testing TPRT with other Cas systems

[1162] Similar experiments to those presented in the previous sections will be carried out using other Cas systems, including Cas9 from *S. aureus* and Cas12a from *L. bacterium* (see FIGs. 2A-2C). If TRPT can also be demonstrated for these Cas variants, the potential editing scope and likelihood of overall success in cells would increase.

Testing TPRT with RT-Cas9 fusion proteins

[1163] A series of commercially available or purifiable RT enzymes will first be evaluated in trans for TPRT activity. In addition to the already tested RT from M-MLV, the RT from Avian Myeloblastosis Virus (AMV), the *Geobacillus stearothermophilus* Group II Intron (GsI-IIC)[41,42], and the *Eubacterium rectale* group II intron (Eu.re.I2)[43,44] will be evaluated. Significantly, the latter two RTs perform TPRT in their natural biological contexts. Where relevant, RNAse inactivating mutations and other potentially beneficial RT enzyme modifications will be tested. Once functional RTs are identified when supplied in trans, each will be evaluated as a fusion protein to Cas9 variants. Both N-terminus and C-terminus fusion orientations will be tested, along with various linker lengths and architectures. Kinetic time course experiments will be used to determine whether TPRT can occur using the RT enzyme in cis. If an RT-Cas9 fusion architecture can be constructed that allows for efficient TPRT chemistry, this will greatly increase the likelihood of functional editing in the context of a cell.

Cas9 targeting with engineered gRNAs in cells

[1164] Candidate engineered gRNAs developed in the previous sub-aims will be evaluated in human cell culture experiments (HEK293) to confirm Cas9 targeting efficiency. Using established indel formation assays employing wild type SpCas9[45], engineered gRNAs will be compared side-by-side with standard gRNAs across 5 or more sites in the human genome. Genome editing efficiency will be characterized by amplicon sequencing in multiplex using the Illumina MiSeq platform housed in the laboratory. It is anticipated that results from this and the preceding sections will generate insights that inform subsequent iterations of the design-build-test cycle, where gRNAs can be optimized for both templating reverse transcription and efficient Cas9 targeting in cells.

[1165] Results of *in vitro* validations are shown in FIGs. 5-7. *In vitro* experiments demonstrated that the nicked non-target DNA strand is flexible and available for priming DNA synthesis, and that the gRNA extension can serve as a template for reverse transcription (see FIG. 5). This set of experiments used 5′-extended gRNAs (designed as shown in FIGs.

3A-3B) with varying length synthesis templates (listed to the left). Fluorescently labeled (Cy5) DNA targets were used as substrates, and were pre-nicked in this set of experiments. The Cas9 used in these experiments is catalytically dead Cas9 (dCas9), so cannot cut DNA but can still bind efficiently. Superscript III, a commercial RT derived from the Moloney-Murine Leukemia Virus (M-MLV), was supplied in trans. First, dCas9:gRNA complexes were formed from purified components. Then, the fluorescently labeled DNA substrate was added along with dNTPs and the RT enzyme. After 1 hour of incubation at 37 C, the reaction products were analyzed by denaturing urea-polyacrylamide gel electrophoresis (PAGE). The gel image shows extension of the original DNA strand to lengths that are consistent with the length of the reverse transcription template. Of note, reactions carried out in the absence of dCas9 produced DNA products of length 51 nucleotides, regardless of the gRNA used. This product corresponds to use of the complementary DNA strand as the template for DNA synthesis and not the RNA (data not shown). Thus, Cas9 binding is required for directing DNA synthesis to the RNA template. This set of *in vitro* experiments closely parallels those shown in FIG. 5, except that the DNA substrate is not pre-nicked, and a Cas9 nickase (SpyCas9 H840A mutant) is used. As shown in the gel, the nickase efficiently cleaves the DNA strand when the standard gRNA is used (gRNA_0, lane 3). Multiple cleavage products are observed, consistent with prior biochemical studies of SpyCas9. The 5′ extension impairs nicking activity (lanes 4-8), but some RT product is still observed. FIG. 7 shows that 3′ extensions support DNA synthesis and do not significantly effect Cas9 nickase activity. Pre-nicked substrates (black arrow) are near-quantitatively converted to RT products when either dCas9 or Cas9 nickase is used (lanes 4 and 5). Greater than 50% conversion to the RT product (red arrow) is observed with full substrates (lane 3). To determine the length and sequence of the RT product, the product band was excised from the gel, extracted, and sequenced. This revealed that RT extended 3 nucleotides into the gRNA core's 3′ terminal hairpin. Subsequent experiments (not shown) demonstrated that these three nucleotides could be changed to match target DNA sequences, so long as complementary changes were made that maintain the hairpin RNA structure.

Potential difficulties and alternatives

[1166] (1) RT does not function as a fusion: molecular crowding and/or unfavorable geometries could encumber polymerase extension by Cas9-fused RT enzymes. First, linker optimization can be tested. Circularly permutated variants of Cas9, which could re-orient the spatial relationship between the DNA primer, gRNA, and RT enzyme, will be evaluated.

Non-covalent RT recruitment strategies as detailed in Aim 2 can be tested. (2) Decreased Cas targeting efficiency by extended gRNA variants: this is most likely to be an issue for 5′-extended gRNAs. Based on structural data[24], Cas9 mutants can be designed and screened to identify variants with greater tolerance to gRNA extension. In addition, gRNA libraries could be screened in cells for linkers that improve targeting activity.

Significance

[1167] These preliminary results establish that Cas9 nickases and extended gRNAs can initiate target-primed reverse transcription on bound DNA targets using a reverse transcriptase supplied in trans. Importantly, Cas9 binding was found to be critically important for product formation. Though perhaps not an absolute requirement for genome editing in cells, further development of the system that incorporates RT enzyme function in cis would significantly increase the likelihood of success in cell-based applications. Achievement of the remaining aspects of this Aim would provide a molecular foundation for carrying out precision genome editing in the context of the human genome.

2. Establish prime editing in human cells.

Background and Rationale

[1168] In the proposed strategy, an engineered RT-Cas9:gRNA complex will introduce mutagenic 3′ DNA flaps at genomic target sites. It is hypothesized that mutagenic 3′ flaps containing a single mismatch will be incorporated by the DNA repair machinery through energetically accessible equilibration with adjacent 5′ flaps, which would be preferentially removed (FIGs. 1C-1D). The DNA replication and repair machineries encounter 5′ ssDNA flaps when processing Okazaki fragments[46] and during long-patch base excision repair (LP-BER)[47]. 5′ flaps are the preferred substrates for the widely expressed flap endonuclease FEN1, which is recruited to DNA repair sites by the homotrimeric sliding clamp complex PCNA[48]. PCNA also serves as a scaffold for simultaneous recruitment of other repair factors including the DNA ligase Lig1[49]. Acting as a 'toolbelt', PCNA accelerates serial flap cleavage and ligation, which is essential to processing the millions of Okazaki fragments generated during every cell division[50,51]. Based on resemblance to these natural DNA intermediates, it is hypothesized that mutagenic strands would be incorporated through equilibration with 5′ flaps, followed by coordinated 5′ flap excision and ligation. Mismatch repair (MMR) should then occur on either strand with equal probability, leading to editing or reversion (FIGs. 1C-1D). Alternatively, DNA replication could occur first and lead directly to the incorporation of the edit in the newly synthesized daughter strand. While the highest

expected yield from this process is 50%, multiple substrate editing attempts could drive the reaction toward completion due to the irreversibility of editing repair.

Preliminary result

[1169] DNA flaps induce site-specific mutagenesis in plasmid model substrates in yeast and HEK cells. To test the proposed editing strategy, studies were initiated with model plasmid substrates containing mutagenic 3′ flaps that resemble the product of TPRT. A dual fluorescent protein reporter was created that encodes a stop codon between GFP and mCherry. Mutagenic flaps encode a correction to the stop codon (FIG. 9A), enabling mCherry synthesis. Thus, mutagenesis efficiency can be quantified by GFP:mCherry ratios. Plasmid substrates were prepared *in vitro* and introduced into yeast (*S. cerevisiae*) or human cells (HEK293). High frequency mutagenesis was observed in both systems (FIG. 9B), and isolated yeast colonies contained either the reverted base, the mutated base, or a mixture of both products (FIG. 9C). Detection of the latter suggests that plasmid replication occurred prior to MMR in these cases, and further suggests that flap excision and ligation precede MMR. This result establishes the feasibility of DNA editing using 3′ mutagenic strands.

- ***Systematic studies with model flap substrates***

[1170] Based on the preliminary results described above, a broader spectrum of flap substrates will be evaluated in HEK cells to infer principles of efficient editing. 3′ ssDNA flaps will be systematically varied to determine the influence of mismatch pairings, the location of the mutagenic nucleotide along the flap, and the identity of the terminal nucleotide (FIG. 9D). Single nucleotide insertions and deletions will also be tested. Amplicon sequencing will be used to analyze editing precision. These results will help inform the design of gRNA reverse transcription templates.

[1171] *In vitro* TPRT on plasmid substrates leads to efficient editing outcomes. TPRT reactions developed in Aim 1 were used to induce mutagenesis within a plasmid substrate. The reaction was carried out on circular DNA plasmid substrates (see FIG. 10). This rules out the possibility of DNA strand dissociation as the mechanism for RT extension in the previous *in vitro* experiments. It also allowed for the testing of DNA repair of flap substrates in cells. A dual-fluorescent reporter plasmid was constructed for yeast (S. cerevisiae) expression. This plasmid encodes GFP (green fluorescent protein) and mCherry (red fluorescent protein) with an intervening stop codon (TGA). Expression of this construct in yeast produces only GFP. The plasmid was used as a substrate for *in vitro* TRT [Cas9(H840A) nickase, engineered gRNA, MLV RT enzyme, dNTPS]. The gRNA extension encodes a mutation to the stop

codon. The flap strand is used for repair of the stop codon and it is anticipated to produce a plasmid that expresses both GFP and mCherry as a fusion protein. Yeast dual-FP plasmid transformants are shown in FIG. 10. Transforming the parent plasmid or an *in vitro* Cas9(H840A) nicked plasmid results in only green GFP expressing colonies. TRT reaction with 5′-extended or 3′-extended gRNAs produces a mix of green and yellow colonies. The latter express both GFP and mCherry. More yellow colonies are observed with the 3′-extended gRNA. A positive control that contains no stop codon is shown as well.

[1172] This result establishes that long double stranded substrates can undergo TPRT, and that TPRT products induce editing in eukaryotic cells.

[1173] Another experiment similar to the foregoing prime editing experiment was carried out, but instead of installing a point mutation in the stop codon, TRT editing installs a single nucleotide insertion (left) or deletion (right) that repairs a frameshift mutation and allows for synthesis of downstream mCherry (see FIG. 11). Both experiments used 3′ extended gRNAs. Individual colonies from the TRT transformations were selected and analyzed by Sanger sequencing (see FIG. 12). Green colonies contained plasmids with the original DNA sequence, while yellow colonies contained the precise mutation designed by the TRT editing gRNA. No other point mutations or indels were observed.

Establish prime editing in HEK cells using RT-Cas9 architectures

[1174] The optimized constructs from previous aims will be adapted for mammalian expression and editing at targeted sites in the human genome. Multiple RT enzymes and fusion architectures will be tested, in addition to adjacent targeting with secondary gRNAs (truncated to prevent nicking). Non-covalent RT recruitment will also be evaluated using the Sun-Tag system[52] and MS2 aptamer system[53]. Indel formation assays will be used to evaluate targeting efficiency with standard gRNAs and RT-Cas9 fusions (as above). Then, for each genomic site, extended gRNAs and RT-Cas9 pairs will be assayed for single nucleotide editing. Editing outcomes will be evaluated with MiSeq.

[1175] Initial experiments in HEK cells were performed using Cas9-RT fusions. Editing by components expressed within cells requires a Cas9(H840A) nickase, a reverse transcriptase (expressed as a fusion or supplied in trans), and an engineered gRNA with a 3′ extension (see FIG. 14). Preliminary studies indicated that the length of the primer binding site within the gRNA extension was important for increasing the efficiency of editing in human cells (see FIG. 15).

Optimize prime editing parameters in HEK cells

[1176] After identifying Cas9-RT architectures that can perform prime editing in cells, the components and design will be optimized to achieve high efficiency editing. The location and nucleotide identity of the encoded point mutation, and the total length of the newly synthesized DNA strand, will be varied to evaluate editing scope and potential limitations. Short insertion and deletion mutations will also be evaluated. Protein expression constructs will be codon optimized. If successful, this would establish efficient prime editing in mammalian cells.

[1177] Preliminary Result. Additional gRNAs were designed to bring the RT enzyme to a higher local concentration at the editing locus, in the event that intramolecular reverse transcription by the fused RT enzyme were not possible. These auxiliary guides are truncated at the 5′ end (14-15 nt spacer), which has previously been shown to prevent Cas9 cutting but retain binding (see FIG. 16). The HEK3 locus was chosen to explore this strategy.

Potential difficulties and alternatives

[1178] 1) gRNA degradation in cells: if extended gRNA termini are truncated in cells, stabilizing secondary structures could be installed, or synthetic gRNAs with stabilizing modifications could be tested. (2) No observed editing in human cells: additional strategies will be explored, including secondary targeting of RT-Cas9 fusions to adjacent genomic sites[54]. In addition, potential directed evolution strategies in E. coli or S. cerevisiae could be explored.

Significance

[1179] If prime editing could be established in experimental cell lines, this would have an immediate impact for basic biomedical research by enabling the rapid generation and characterization of a large number of point mutations in human genes. The generality of the method, and its orthogonal editing window with respect to base editors, would provide an approach to installing many currently inaccessible mutations. Moreover, if prime editing could be optimized for high efficiency and product purity, its potential applicability to correcting disease mutations in other human cell types would be significant.

[1180]

3. Achieve site-specific editing of pathogenic mutations in cultured human cells.

Background and Rationale.

[1181] A large number of pathogenic mutations cannot be corrected by current base editors due to PAM restrictions, or a need for transversion or indel mutation correction. With prime editing, all transitions and transversions are theoretically possible, as may be small insertions

and deletions. Moreover, in relation to the PAM, the prime editing window (anticipated -3 to +4) is distinct from that of base editors (-18 to -12) (FIG. 13). Mendelian conditions not currently correctable by base editors include: (1) the sickle cell disease Glu6Val founder mutation in hemoglobin beta (requires A•T to T•A transversion); (2) the most common Wilson's disease variant His1069Gln in ATP7B (requires G•C to T•A transversion); and (3) the ΔPhe508 mutation in *CFTR* that causes cystic fibrosis (requires 3-nucleotide insertion). Each of these targets contains an appropriately positioned PAM for SpCas9 targeting and prime editing.

Preliminary Results.

- ***T to A editing in HEK3 cells is not achievable by current base editing but is achievable by TRPT editing (see FIGs. 17A-17C).***

[1182] FIG. 17A shows a graph displaying the % T to A conversion at the target nucleotide after transfection of components in human embryonic kidney (HEK) cells. This data presents results using an N-terminal fusion of wild type MLV reverse transcriptase to Cas9(H840A) nickase (32-amino acid linker). Editing efficiency was improved dramatically when the length of the primer binding site is extended from 7 nucleotides to 11 or 12 nucleotides. Additionally, the auxiliary guide A, which is positioned just upstream of the editing locus (see FIG. 16), significantly improves editing activity, particularly for shorter length primer binding sites. Editing efficiency was quantified by amplicon sequencing using the Illumina MiSeq platform. FIG. 17B also shows % T to A conversion at the target nucleotide after transfection of components in human embryonic kidney (HEK) cells, but this data presents results using a C-terminal fusion of the RT enzyme. Here, the auxiliary guide A does not have as much of an effect, and editing efficiency is overall higher. FIG. 17C shows data presenting results using an N-terminal fusion of wild type MLV reverse transcriptase to Cas9(H840A) nickase similar to that used in FIG. 17A; however the linker between the MLV RT and Cas9 is 60 amino acids long instead of 32 amino acids.

- ***T to A editing at HEK3 site by TRPT editing results displays high purity.***

[1183] FIG. 18 shows the output of sequencing analysis by high-throughput amplicon sequencing. The output displays the most abundant genotypes of edited cells. Of note, no major indel products are obtained, and the desired point mutation (T to A) is cleanly installed without bystander edits. The first sequence shows the reference genotype. The top two products are the starting genotype containing an endogenous polymorphism (G or A). The bottom two products represent the correctly edited genotypes.

- ***MLV RT mutants improve editing.***

[1184] Mutant reverse transcriptases, described in Baranauskas, et al (doi:10.1093/protein/gzs034), were tested as C-terminal fusions to the Cas9(H840A) nickase for target nucleotide editing in human embryonic kidney (HEK) cells. Cas9-RT editor plasmid was co-transfected with a plasmid encoding a 3′-prime editing guide RNA that templates reverse transcription. Editing efficiency at the target nucleotide (blue bars) is shown alongside indel rates (orange bars) in FIG. 19. WT refers to the wild type MLV RT enzyme. The mutant enzymes (M1 through M4) contain the mutations listed to the right. Editing rates were quantified by high throughput sequencing of genomic DNA amplicons.

Complementary strand nicking with a second gRNA improves editing.

[1185] This experiment evaluates editing efficiency of the target nucleotide when a single strand nick is introduced in the complementary DNA strand in proximity to the target nucleotide, with the hypothesis being that this would direct mismatch repair to preferentially remove the original nucleotide and convert the base pair to the desired edit. The Cas9(H840A)-RT editing construct was co-transfected with two guide RNA encoding plasmids, one of which templates the reverse transcription reaction, while the other targets the complementary DNA strand for nicking. Nicking at various distances from the target nucleotide was tested (orange triangles) (see FIG. 20). Editing efficiency at the target base pair (blue bars) is shown alongside the indel formation rate (orange bars). The “none” example does not contain a complementary strand nicking guide RNA. Editing rates were quantified by high throughput sequencing of genomic DNA amplicons.

[1186] FIG. 21 shows processed high throughput sequencing data showing the desired T to A transversion mutation and general absence of other major genome editing byproducts.

[1187] Scope. The potential scope for the new editing technology is shown in FIG. 13 and is compared to deaminase-mediated base editor technologies. Previously developed base editors target a region ~15 ± 2 bp upstream of the PAM. By converting target C or A nucleotides to T or G, respectively, previously developed base editors enable all transition mutations (A:T to G:C conversions). However, previously developed base editors are unable to install transversion mutations (A to T, A to C, G to T, G to C, T to A , T to G, C to A, C to G). Moreover, if there are multiple target nucleotides in the editing window, additional undesired edits can result.

[1188] The new prime editing technology could theoretically install any nucleotide and base pair conversion, and potentially small insertion and deletion edits as well. With respect to the

PAM, prime editing windows start at the site of DNA nicking (3 bases upstream of the PAM) and end at an as-of-yet undetermined position downstream of the PAM. Of note, this editing window is distinct from that of deaminase base editors. Because the TPRT systems performs editing using DNA polymerase enzymes, it potentially has all of their benefits including generality, precision, and fidelity.

Correct pathogenic mutations in patient-derived cell lines.

[1189] Cell lines harboring the relevant mutations (sickle cell disease: CD34+ hematopoietic stem cells; Wilson's disease: cultured fibroblasts; cystic fibrosis: cultured bronchial epithelia) will be obtained from ATCC, the Coriell Biobank, or collaborating Harvard/Broad affiliate laboratories. Editing efficiency will be evaluated by high throughput sequencing, and the efficacy of the corrected genotype will be tested using phenotypic assays (hemoglobin HPLC, ATP7B immunostaining, and *CFTR* membrane potential assays).

Characterize off-target editing activity.

[1190] Potential off-target editing will be screened with established methods such as GUIDE-seq[55] and CIRCLE-seq[56] using target gRNAs paired with wild type Cas9. If potential off-targets are identified, these loci will be probed in TPRT edited cells to identify true off-target editing events.

Potential difficulties and alternatives.

[1191] (1) Low editing efficiency: prime editor (PE)s may require optimization for each target. In this case, gRNA libraries can be tested to identify the highest functioning variants for specific applications. RT-Cas fusion expression and nuclear localization can be optimized. Liposomal RNP delivery could be used to limit off-target editing.

Upcoming experiments.

[1192] Optimization of gRNA designs can be achieved by further exploration of the primer binding site length and extension of synthesis template. Testing scope and generality will include different nucleotide conversions, small insertions and deletions, as well as, different editing positions with respect to PAM, and multiple sites in the human genome. Optimization of RT component will include exploring mutations in MLV RT to enhance activity (Rnase H inactivation, increase primer-template binding affinity, adjustments to processivity), and new RT enzymes (group II intro RTs, other retroviral RTs).

Significance.

[1193] Myriad genetic disorders result from single nucleotide changes in individual genes. Developing the genome editing technology described here, and applying it in disease-relevant

cell types, would establish a foundation for translation to the clinic. For some diseases, such as Sickle Cell Disease, a single point mutation represents the dominant genotype throughout the population. However, for many other genetic disorders, a large heterogeneity of different point mutations within a single gene is observed throughout the patient population, each of which gives rise to a similar disease phenotype. Therefore, as a general genome editing method that could in theory target a large number of such mutations, this technology could provide enormous potential benefit to many of these patients and their families. If proof of principle for these applications could be established in cells, it would establish the foundation to studies in animal models of disease.

Advantages

[1194] Precision: the desired edit is encoded directed in nucleic acid sequence. Generality: in theory, could be possible to make any base pair conversion, including transversion edits, as well as small insertions or deletions. There is a distinct editing window from that of base editors with respect to Cas9 protospacer adjacent motif (PAM) sequence. This method achieves many of the editing capabilities of homology-directed repair (HDR), but without the major limitations of HDR (inefficient in most cell types, and is usually accompanied by an excess of undesired byproducts such as indels). Also, it does not make double-stranded DNA breaks (DSBs, so few indels, translocations, large deletions, p53 activation, etc.

[1195]

EXAMPLE 2 – ERROR-PRONE PRIME EDITING (PE)

[1196] Prime editing (PE) systems described herein may also be used in conjunction with an error-prone reverse transcriptase enzyme to install mutations in a genome.

[1197] An embodiment is depicted in FIG. 22, which is a schematic of an exemplary process for conducting targeted mutagenesis with an error-prone reverse transcriptase on a target locus using a nucleic acid programmable DNA binding protein (napDNAbp) complexed with an extended guide RNA. This process may be referred to as an embodiment of prime editing for targeted mutagenesis. The extended guide RNA comprises an extension at the 3′ or 5′ end of the guide RNA, or at an intramolecular location in the guide RNA. In step (a), the napDNAbp/gRNA complex contacts the DNA molecule and the gRNA guides the napDNAbp to bind to the target locus to be mutagenized. In step (b), a nick in one of the strands of DNA of the target locus is introduced (e.g., by a nuclease or chemical agent), thereby creating an available 3′ end in one of the strands of the target locus. In certain embodiments, the nick is created in the strand of DNA that corresponds to the R-loop strand,

i.e., the strand that is not hybridized to the guide RNA sequence. In step (c), the 3′ end DNA strand interacts with the extended portion of the guide RNA in order to prime reverse transcription. In certain embodiments, the 3′ ended DNA strand hybridizes to a specific RT priming sequence on the extended portion of the guide RNA. In step (d), an error-prone reverse transcriptase is introduced which synthesizes a mutagenized single strand of DNA from the 3′ end of the primed site towards the 3′ end of the guide RNA. Exemplary mutations are indicated with an asterisk "*". This forms a single-strand DNA flap comprising the desired mutagenized region. In step (e), the napDNAbp and guide RNA are released. Steps (f) and (g) relate to the resolution of the single strand DNA flap (comprising the mutagenized region) such that the desired mutagenized region becomes incorporated into the target locus. This process can be driven towards the desired product formation by removing the corresponding 5′ endogenous DNA flap that forms once the 3′ single strand DNA flap invades and hybridizes to the complementary sequence on the other strand. The process can also be driven towards product formation with second strand nicking, as exemplified in FIG. 1F. Following endogenous DNA repair and/or replication processes, the mutagenized region becomes incorporated into both strands of DNA of the DNA locus.

EXAMPLE 3 –TRINUCLEOTIDE REPEAT CONTRACTION WITH PE

[1198] The prime editing system or prime editing (PE) system described herein may be used to contract trinucleotide repeat mutations (or "triplet expansion diseases") to treating conditions such as Huntington's disease and other trinucleotide repeat disorders. Without wishing to be bound by theory, triplet expansion is caused by slippage during DNA replication or during DNA repair synthesis. Because the tandem repeats have identical sequence to one another, base pairing between two DNA strands can take place at multiple points along the sequence. This may lead to the formation of "loop out" structures during DNA replication or DNA repair synthesis. This may lead to repeated copying of the repeated sequence, expanding the number of repeats. Additional mechanisms involving hybrid RNA:DNA intermediates have been proposed. Prime editing may be used to reduce or eliminate these triplet expansion regions by deletion one or more or the offending repeat codon triplets. In an embodiment of this use, FIG. 23, provides a schematic of a PEgRNA design for contracting or reducing trinucleotide repeat sequences with prime editing.

[1199] Thus, prime editing may be able to be used to correct any trinucleotide repeat disorder, including, Huntington's disease, Fragile X syndrome, and Friedreich's ataxia.

[1200] The most common trinucleotide repeat contains CAG triplets, though GAA triplets (Friedreich's ataxia) and CGG triplets (Fragile X syndrome) also occur. The CAG triplets code for glutamine (Q), thus, CAG repeats result in polyglutamine tracts in the coding regions of diseased proteins. This particular class of trinucleotide repeat disorders are also called "polyglutamine (PolyQ) diseases." Other trinucleotide repeats can cause alterations in gene regulation and are referred to as "non-polyglutamine diseases." Inheriting a predisposition to expansion, or acquiring an already expanded parental allele, increases the likelihood of acquiring the disease. Pathogenic expansions of trinucleotide repeats could be corrected using prime editing.

[1201] Prime editing may be implemented to contract triplet expansion regions by nicking a region upstream of the triplet repeat region with the prime editor comprising a PEgRNA appropriated targeted to the cut site. The prime editor then synthesizes a new DNA strand (ssDNA flap) based on the PEgRNA as a template (i.e., the edit template thereof) that codes for a healthy number of triplet repeats (which depends on the particular gene and disease). The newly synthesized ssDNA strand comprising the healthy triplet repeat sequence also is synthesized to include a short stretch of homology (i.e., the homology arm) that matches the sequence adjacent to the other end of the repeat (red strand). Invasion of the newly synthesized strand, and subsequent replacement of the endogenous DNA with the newly synthesized ssDNA flap, leads to a contracted repeat allele.

EXAMPLE 4 – PEPTIDE TAGGING WITH PE

[1202] The prime editing systems (i.e., PE systems) described herein may also be used to introduce various peptide tags into protein coding genes. Such tags can include *HEXA*histidine tags, FLAG-tag, V5-tag, GCN4-tag, HA-tag, Myc-tag and others. This approach may be useful in applications such as protein fluorescent labeling, immunoprecipitation, immunoblotting, immunohistochemistry, protein recruitment, inducible protein degrons, and genome-wide screening. Embodiments are depicted in FIGs. 25 and 26.

[1203] FIG. 25 is a schematic showing gRNA design for peptide tagging genes at endogenous genomic loci and peptide tagging with TPRT genome editing (i.e., prime editing). The FlAsH and ReAsH tagging systems comprise two parts: (1) a fluorophore-biarsenical probe, and (2) a genetically encoded peptide containing a tetracysteine motif, exemplified by the sequence FLNCCPGCCMEP (SEQ ID NO: 1). When expressed within cells, proteins containing the tetracysteine motif can be fluorescently labeled with fluorophore-arsenic probes (see ref: J. Am. Chem. Soc., 2002, 124 (21), pp 6063–6076.

DOI: 10.1021/ja017687n). The "sortagging" system employs bacterial sortase enzymes that covalently conjugate labeled peptide probes to proteins containing suitable peptide substrates (see ref: Nat. Chem. Biol. 2007 Nov;3(11):707-8. DOI: 10.1038/nchembio.2007.31). The FLAG-tag (DYKDDDDK (SEQ ID NO: 2)), V5-tag (GKPIPNPLLGLDST (SEQ ID NO: 3)), GCN4-tag (EELLSKNYHLENEVARLKK (SEQ ID NO: 4)), HA-tag (YPYDVPDYA (SEQ ID NO: 5)), and Myc-tag (EQKLISEEDL (SEQ ID NO: 6)) are commonly employed as epitope tags for immunoassays. The pi-clamp encodes a peptide sequence (FCPF) (SEQ ID NO: 622) that can by labeled with a pentafluoro-aromatic substrates (ref: Nat. Chem. 2016 Feb;8(2):120-8. doi: 10.1038/nchem.2413).

[1204] FIG. 26 shows precise installation of a His6-tag and a FLAG-tag into genomic DNA. A guide RNA targeting the HEK3 locus was designed with a reverse transcription template that encodes either an 18-nt His-tag insertion or a 24-nt FLAG-tag insertion. Editing efficiency in transfected HEK cells was assessed using amplicon sequencing. Note that the full 24-nt sequence of the FLAG-tag is outside of the viewing frame (sequencing confirmed full and precise insertion).

[1205]

EXAMPLE 5 – PREVENTION OR TREATMENT OF PRION DISEASE WITH PE

[1206] This invention could help address the problem of prion disease in humans, livestock, and wildlife. No previously described editing strategy is efficient and clean enough to install protective mutations or to reliably knock down *PRNP*. Cas9 nuclease and HDR can be used but will generate mostly a mixture of *PRNP* indel variants some of which are thought to be pathogenic. Moreover, HDR does not work in most types of cells. Prime editing is reliable and efficient at installing both types of mutations without generating an excess of double-stranded DNA breaks or resulting indels.

[1207] This invention describes how to install a protective mutation in *PRNP* that prevents or halts the progression of prion disease. This site is conserved in mammals, so in addition to treating human disease it could also be used to generate cows and sheep that are immune to prion disease, or even help cure wild populations of animals that are suffering from prion disease. Prime editing has already been used to achieve ~25% installation of a naturally occurring protective allele in human cells, and previous mouse experiments indicate that this level of installation is sufficient to cause immunity from most prion diseases. This method is the first and potentially only current way to install this allele with such high efficiency in most cell types. Another possible strategy for treatment is to use prime editing to reduce or

eliminate the expression of *PRNP* by installing an early stop codon in the gene. Many researchers predict that doing so would treat the disease.

[1208] Three potential therapeutic strategies include prime editing to reduce expression of PrP. This goal may be accomplished by the introduction of mutations that cause a premature stop codon in *PRNP*, eliminate the start codon, mutate or delete essential amino acids codons, introduce or remove splice sites to generate an aberrant transcript, or alter regulatory elements that reduce transcript levels. Prime editing to eliminate disease mutations. Many variants of *PRNP* have been described (ncbi.nlm.nih.gov/pmc/articles/PMC6097508/#b154-ndt-14-2067) that lead to an increased likelihood of contracting disease. Each known variant could be reversed using prime editing, since prime editing can make all possible types of point mutations, local insertions, and local deletions. Prime editing to introduce one or more protective mutations into *PRNP* that disrupt prion formation and/or transmission. For example, G127V in the human *PRNP* gene has been demonstrated to protect against many forms of prion disease (ncbi.nlm.nih.gov/pmc/articles/PMC4486072/). This mutation was later described to interfere with prion formation by preventing formation of stable beta sheets and dimers (ncbi.nlm.nih.gov/pubmed/30181558, ncbi.nlm.nih.gov/pubmed/26906032). In addition to the introduction of single nucleotide polymorphisms, the insertion or deletion of sequences in *PRNP* that would interfere with prion formation could also be used to protect from or treat prion disease.

[1209] The third therapeutic strategy is particularly advantageous because the introduction of protective variants could confer a benefit even when a relatively small number of cells experience the edit. Furthermore, the introduction of protective variants, especially those naturally occurring in human populations such as G127V, would not be expected to have any detrimental consequences, while reducing expression of prion protein as in strategy 1 could have some detrimental phenotypes, as have been documented in *PRNP* knock-out mice (ncbi.nlm.nih.gov/pmc/articles/PMC4601510/, ncbi.nlm.nih.gov/pmc/articles/PMC2634447/).

[1210] It has previously been demonstrated that mice expressing a ratio of approximately 2:1 of the wild type human prion protein: the protective G127V variant of the human prion protein (approximately 33% expression of the protective variant) were entirely immune to most tested forms of prion disease and were also resistant to variant Creutzfeldt-Jakob disease (vCJD), the human disease transmitted from bovine spongiform encephalopathy

(BSE, or mad cow disease)[91]. Mice that only expressed the protective G127V variant were entirely immune to all tested prion disease challenges, including vCJD.

[1211] It is demonstrated herein that the protective G127V mutation can be efficiently installed in human cells in tissue culture using prime editing (see FIG. 27).

[1212] Informed by these results, three settings are described in which *PRNP* editing could be used. One setting *PRNP* editing can be used is prime editing in human patients to prevent or treat prion disease. A second setting *PRNP* editing can be used is prime editing in livestock to prevent the occurrence and spread of prion disease. Both cow and sheep livestock have experienced sporadic occurrence of prion disease caused by the protein generated by the *PRNP* gene. In addition to the debilitating and deadly disease suffered by the animal, these cases are also economically devastating, in part due to the care that must be taken to prevent the spread of the extremely infectious disease. A single dairy cow in the state of Washington tested positive for BSE in December of 2003, which led to a projected loss of 2.8-4.2 billion dollars in beef sales the following year bookstore.ksre.ksu.edu/pubs/MF2678.pdf). The *PRNP* gene is highly conserved in mammals. Introducing a *PRNP* mutation such as G127V into the livestock germline could eliminate the occurrence of BSE or scrapie, the manifestation of prion disease in sheep. A third setting *PRNP* editing can be used is prime editing in wildlife could prevent the spread of wild prion disease. Currently, cervid populations including deer, elk, and moose in North America are suffering from chronic wasting disease (CWD), a manifestation of prion disease caused by PNRP in these species. The occurrence has been reported to be as high as 25% in some populations (cdc.gov/prions/cwd/occurrence.html). CWD has also been reported in Norway, Finland, and South Korea. It is not yet known whether the disease is transmissible from these species to humans (cdc.gov/prions/cwd/transmission.html) or livestock. The introduction of *PRNP* mutations such as G127V in the germline of these species could protect them from CWD and reduce the risk of transmission to other species including humans.

[1213] This method could be used to treat Creutzfeldt-Jakob Disease (CJD), kuru, Gerstmann-Sträussler- Scheinker disease, fatal familial insomnia (FFI), bovine spongiform encephalopathy (BSE; mad cow disease), scrapie (in sheep), and chronic wasting disease (CWD; in deer, elk, and moose).

[1214] The method would need to be combined with a delivery methodology for embryos or adult neurons, such as microinjection, lipid nanoparticles, or AAV vectors.

EXAMPLE 6 – RNA TAGGING AND MANIPULATION USING PE

[1215] A new method for the insertion of motifs into genetic sequences that tag or otherwise manipulate RNA within mammalian, eukaryotic, and bacterial cells is described herein. While it is estimated that only 1% of the human genome encodes proteins, virtually all of the genome is transcribed at some level. It is an open question how much of the resulting non-coding RNA (ncRNA) plays a functional role, let alone what the roles of most of these putative RNAs are. "Tagging" of these RNA molecules via the insertion of a novel RNA-encoding sequence with a useful property into genes of interest is a useful method for studying the biological functions of RNA molecules in cells. It can also be useful install tags onto protein-encoding mRNAs as a means to perturb and thus better understand how mRNA modifications can affect cellular function. For instance, a ubiquitous natural RNA tag – polyadenylation – is used by cells to affect transport of mRNA into the cytoplasm. Different types of polyadenylation signals result in different transport rates and different mRNA lifespans and – thus – differences in the levels to which the encoded protein is expressed.

[1216] A common approach for expressing tagged RNAs within cells is to exogenously introduce a synthetic construct using either (i) transient plasmid transfection that produces a short-term burst of gene expression, often at supraphysiologic levels; or (ii) permanent integration of the tagged RNA gene into the genome (at random sites) using lentiviral integration or transposons, which enables prolonged expression. Both of these approaches are limited by production of altered expression levels, and by the absence of natural mechanisms that regulate the expression or activity of the gene. An alternative strategy is to directly tag a gene of interest at its endogenous locus using homology-directed repair (HDR) of double-stranded DNA breaks induced by Cas9 or other targeted DNA nucleases. While this approach enables the generation of a wide range of endogenously tagged genes, HDR is markedly inefficient and so requires significant screening to identify the desired clonal population of cells that have been successfully tagged. Moreover, HDR is typically very inefficient or entirely inactive in a large number of cell types, most notably in post-mitotic cells. The low efficiency of HDR is further complicated by the generation of undesired indel products, would could be especially problematic in the case of RNA genes as they might lead to the production of an RNA whose activities interfere with the function of normal alleles. Finally, researchers often need to screen various tagging positions within an RNA molecule to achieve optimal performance. Combined, these drawbacks make HDR a less desirable method for installation of tags in RNA.

[1217] Prime editing is a new genome editing technology that enables targeted editing of genomic loci via the transfer of genetic information from RNA to DNA. Using prime editing, RNA genes could be tagged with a variety of components such as RNA aptamers, ribozymes, or other RNA motifs. Prime editing has the potential to be faster, cheaper, and effective in a greater variety of cell types by comparison to HDR strategies. As such, the described invention represents a novel, useful, and non-obvious tool for investigating the biology of RNA genes in health and disease. A new method for the insertion of RNA motifs into genetic sequences that tag or otherwise manipulate RNA using prime editors (PEs) is described herein. PEs are capable of site-specifically inserting, mutating, and/or deleting multiple nucleotides at a desired genomic locus that is targetable by a CRISPR/Cas system. PEs are composed of fusions between Cas9 nuclease domains and reverse transcriptase domains. They are guided to their genomic target by engineered PEgRNAs (prime editing guide RNAs), which contain a guide spacer portion for DNA targeting, as well as a template for reverse transcription that encodes the desired genome edit (see FIG. 28A). It is envisioned that PE can be used to insert motifs that are functional at the RNA level (hereafter RNA motifs) to tag or otherwise manipulate non-coding RNAs or mRNAs. These motifs could serve to increase gene expression, decrease gene expression, alter splicing, change post-transcriptional modification, affect the sub-cellular location of the RNA, enable isolation or determination of the intra- or extra-cellular location of the RNA (using, for instance, fluorescent RNA aptamers such as Spinach, Spinach2, Baby Spinach, or Broccoli), recruit endogenous or exogenous protein or RNA binders, introduce sgRNAs, or induce processing of the RNA, by either self-cleavage or RNAses (see FIG. 28B). Due to the flexibility of prime editing, it is not possible to provide a comprehensive list of RNA motifs that could be installed within the genome. A series of examples are shown here that broadly illustrate the predicted scope of PE-installed RNA motifs that could be used to tag RNA genes. It is currently not possible to efficiently and fairly cleanly make these changes in most types of cells (including the many that do not support HDR) using any other reported genome editing method besides PE.

[1218] Gene expression could be affected by encoding a 3′ untranslated region (UTR) that results in changes in nuclear transport or retention or mRNA lifespan. For instance, the polyA tail from polyomavirus simian virus 40 (SV40) has additional helper sequences that enable efficient transcription termination and can increase gene expression relative to other 3′ UTRs[57, 58]. Example sequence of SV40 polyA tail:

SV40 POLYA TAIL	AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT TGTGGTTTGTCCAAACTCATCAATGTATCTTA (SEQ ID NO: 331)

[1219] Post-translational modification signals, besides polyadenylation signals, could also be encoded by PE. These include signals incorporate N6-methyladenosine, N1-methyladenosine, 5-methylcytosine, and pseudouridine modifications[59]. By using PE to include sequences bound by enzymes that write or remove these modifications within an RNA transcript, it would be possible to induce their writing or erasing. This could be used as a tool to study the effects of these markers, to induce cellular differentiation, affect stress responses, or, given the function of these markers are as yet underexplored, affect targeted cells in other fashions.

[1220] PE could encode mutations that affect subcellular localization. For instance, incorporation of tRNA-Lys within an mRNA can theoretically result in transport to the mitochondria[60], while various 3′ UTRs can result in nuclear retention or transport[61].

[1221] Examples:

[1222] SV40 polyA signal results in transport.

SV40 POLYA TAIL	AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT TGTGGTTTGTCCAAACTCATCAATGTATCTTA (SEQ ID NO: 331)

[1223] U1 snRNA 3′ box results in retention.

U1 SNRNA 3′ BOX	TTCATTCAGCAAGTTCAGAGAAATCTGAACTTGCTGGATTTTTG GAGCAGGGAGATGGAATAGGAGCTTGCTCCGTCCACTCCACGC ATCGACCTGGTATTGCAGTACCTCCAGGAACGGTGCACCCACTT TCTGGAGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTT TTTGTTT GGTTTGTGTCTTGGTTGGCGTCTTAA (SEQ ID NO: 625)

[1224] Determining the sub-cellular localization of endogenous RNA can be challenging and requires the addition of exogenous, fluorescently-tagged nucleotide probes, as in the case of FISH, or time-consuming and potentially inaccurate cell fractionation followed by RNA detection. Encoding a probe within the endogenous RNA would obviate many of these issues. One example would be to encode a fluorescent RNA aptamer, such as Spinach[62] or Broccoli within an endogenous RNA, thereby visualizing the presence of that RNA via addition of a small molecule proto-fluorophore.

[1225] Broccoli aptamer:

BROCCOLI APTAMER	GAGACGGTCGGGTCCAGATATTCGTATCTGTCGAGTAGAGTGTG GGCTC (SEQ ID NO: 357)

[1226] PE could insert or remove sequences that encode RNA that are recognized by RNA binding proteins, affecting RNA stability, expression, localization, or modification (for instance, see proteins listed[63]).

[1227] PE could insert sequences that encode sgRNAs within the genome, as a viral or cancer defense mechanism. Similarly, it could be used to insert microRNAs (e.g., pre-microRNAs) to direct silencing of targeted genes.

[1228] PE could insert sequences resulting in processing of the RNA, either by itself, or by external factors, either as a therapy or tool for studying the function of various portions of the RNA. For instance, the HDV ribozyme[64], when included within an RNA sequence, results in processing of the RNA immediately 5′ of the ribozyme, while the hammerhead ribozyme cleaves prior to the third stem within the ribozyme[65]. Other self-cleaving ribozymes include pistol[66], hatchet[66], hairpin[67], *Neuropora* Varkud satellite[68], glmS[69], twister[70], and twister sister[66]. These sequences could include wild-type or engineered or evolved versions of ribozymes. The majority of these ribozymes could have different sequences depending on the region of RNA into which they were associated, depending on where the ribozyme cut site is located. Sequences that would direct the processing of the RNA by external factors, such as sequence specific RNAses[71], RNAses that recognize specific structures[72] – such as Dicer[73] and Drosha[74], could also be achieved.

[1229] HDV ribozyme:

HDV RIBOZYME	GGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAA CATGCTTCGGCATGGCGAATGGGAC (SEQ ID NO: 365)

[1230] References for Example 6

[1231] The following references are incorporated herein by reference in their entireties.

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EXAMPLE 7 – GENERATION OF GENE LIBRARIES WITH PE

[1232] A new method for the cellular generation of highly sophisticated libraries of protein- or RNA-coding genes with defined or variable insertions, deletions, or defined amino acid/nucleotide conversions, and their use in high-throughput screening and directed evolution is described herein. The references cited in the Example are based from the list of references included at the end of this Example.

[1233] The generation of variable genetic libraries has most commonly been accomplished through mutagenic PCR[1]. This method relies on either using reaction conditions that reduce the fidelity of DNA polymerase, or using modified DNA polymerases with higher mutation rates. As such, biases in these polymerases are reflected in the library product (e.g. a preference for transition mutations versus transversions). An inherent limitation of this approach to library construction is a relative inability to affect the size of the gene being varied. Most DNA polymerases have extremely low rates of indel mutations[2] (insertions or deletions), and most of these will result in frameshift mutations in protein-coding regions, rendering members of the library unlikely to pass any downstream selection. Additionally, biases in PCR and cloning can make it difficult to generate single libraries consisting of genes of different sizes. These limitations can severely limit the efficacy of directed evolution to enhance existing or engineer novel protein functions. In natural evolution, large changes in protein function or efficacy are typically associated with insertion and deletion mutations that are unlikely to occur during canonical library generation for mutagenesis. Furthermore, these mutations most commonly occur in regions of the protein in question that are predicted to form loops, as opposed to the hydrophobic core. Thus, most indels generated using a traditional unbiased approach are likely to either be deleterious or ineffective.

[1234] Libraries that could bias such mutations to the sites within the protein where they would be most likely to be beneficial, i.e. loop regions, would have a significant advantage over traditional libraries given that all libraries access only a fraction of the possible mutation space. Finally, although it is possible to generate genetic libraries with site-specific indel mutations through multistep PCR and clonal assembly using NNK primers or via DNA

shuffling, these libraries cannot undergo additional rounds of 'indelgenesis' in continual evolution. Continuous evolution is a type of directed evolution with minimal user intervention. One such example is PACE[3]. Because continuous evolution occurs with minimal user intervention, any increase in library diversity during the evolution must occur using the native replication machinery. As such, although libraries of genes with inserted or removed codons as specific loci can be generated and screened in PACE, additional rounds of 'indelgenesis' are not possible.

[1235] It is envisioned that the programmability of prime editing (PE) can be leveraged to generate highly sophisticated, programmed genetic libraries for use in high-throughput screening and directed evolution (see FIG. 29A). PE can insert, change or remove defined numbers of nucleotides from specified genetic loci using information encoded in a prime editing guide RNA (PEgRNA) (see FIG. 29B). This enables the generation of targeted libraries with one or more amino acids inserted or removed from the loop regions wherein mutations are most likely to give rise to changes in function, without background introduction of nonfunctional frameshift mutations (see FIG. 29C). PE can be used to install specific sets of mutations without regard for biases inherent in either DNA polymerase or the sequence being mutated.

[1236] For instance, while converting a CCC codon to a stop codon would be an unlikely occurrence via canonical library generation because it would require three consecutive mutations, including two transversions, PE could be used to convert any given, targeted codon to a TGA stop codon in one step. They could also be used to install programmed diversity at given positions, for instance by incorporating codons encoding any hydrophobic amino acid at a given site, while not encoding any others. Furthermore, because of the programmability of PE, multiple PEgRNAs could be utilized to generate multiple different edits at multiple sites simultaneously, enabling the generation of highly programmed libraries (see FIG. 29D). Additionally, it is possible to use reverse transcriptases with lower fidelity to generate regions of mutagenesis within an otherwise invariable library (such as the HIV-I reverse transcriptase[4] or Bordetella phage reverse transcriptase[5]).

[1237] The possibility of iterative rounds of PE on the same site is also envisioned, allowing–for instance–the repeated insertion of codons at a single site. Finally, it is envisioned that all of the above described approaches can be incorporated into continual evolution, enabling the generation of novel in situ evolving libraries (see FIG. 30). They could also be used to construct these libraries within other cell types where it would otherwise be difficult to

assemble large libraries, for instance within mammalian cells. Generation of PE-encoding bacterial strains that have been optimized for directed evolution would be a useful additional tool for the identification of proteins and RNAs with improved or novel functionality. All of these uses of PE are non-obvious due to the novel nature of PEs. In conclusion, library generation via PE would be a highly useful tool in synthetic biology and directed evolution, as well as for high-throughput screening of protein and RNA combinatorial mutants.

Competing Approaches

[1238] The chief method by which diverse libraries are currently generated is by mutagenic PCR[1], described above. Insertions or deletions can be introduced via degenerated NNK primers at defined sites during PCR, although introducing such mutations at multiple sites requires multiple rounds of iterative PCR and cloning before constructing a more diverse library via mutagenic PCR, rendering the method slow. An alternative, complementary method is DNA shuffling, where fragments of a library of genes generated via DNase treatment are introduced into a PCR reaction without primers, resulting in the annealing of different fragments to each other and the rapid generation of more diverse libraries than via mutagenic PCR alone[6]. Although this approach can theoretically generate indel mutations, it more often results in frameshift mutations that destroy gene function. Furthermore, DNA shuffling requires a high degree of homology between gene fragments. Both of these methods must be done *in vitro,* with the resulting library transformed into cells, while libraries generated by PE can be constructed in situ, enabling their use in continual evolution. While libraries can be constructed *in situ* through *in vivo* mutagenesis, these libraries rely on the host cellular machinery and exhibit biases against indels. Similarly, although traditional cloning methods can be used to generate site-specific mutational profiles, they cannot be used *in situ* and are generally assembled one at a time *in vitro* before being transformed into cells. The efficiency and broad functionality of PE in both prokaryotic and eukaryotic cell types further suggests that these libraries could be constructed directly in the cell type of interest, as opposed to being cloned into a model organism such as *E. coli* and then transferred into the cell or organism of interest. Another competing approach for targeted diversification is automated multiplex genome engineering, or MAGE, wherein multiple single-stranded DNA oligonucleotides can be incorporated within replication forks and result in programmable mutations[7]. However, MAGE requires significant modification of the host strain and can lead to a 100-fold increase in off-target or background mutations[8], whereas PE is more highly programmed and anticipated to result in fewer off-target effects. Additionally, MAGE has not

been demonstrated in a wide variety of cell types, including mammalian cells. Prime editing is a novel and non-obvious complementary technique for library generation.

Examples of PE in Directed Evolution to Construct Gene Libraries

[1239] In one example, PE can be used in a directed evolution experiment to introduce protein variants into gene libraries during a continual evolution experiment using PACE, permitting iterative accumulation of both point mutations and indels in a manner not possible via traditional approaches. It has already been shown that PE can site-specifically and programmably insert nucleotides into a genetic sequence in *E. coli.* In the outlined directed evolution, it is proposed to identify monobodies with improved binding to a specific epitope via a modified two-hybrid protein:protein binding PACE selection. Specific and highly variable loops within these monobodies contribute significantly to affinity and specificity. Improved monobody binding might be obtained rapidly in PACE by varying the length and composition of these loops in a targeted fashion. However, varying sequence length is not an established functionality of PACE. While library of varied loop sizes might be used as a starting point for PACE, no subsequent improvements to length would arise throughout the PACE selection, barring access to beneficial synergistic combinations of point mutations and indel mutations. Introducing PE to the PACE selection would enable the *in situ* generation and evolution of monobodies with varying loop lengths. To do so, it is envisioned the introduction of an additional PE plasmid to the host *E. coli* strain, encoding the PE enzyme and one or more PEgRNAs. Expression of PE enzyme and PEgRNAs would be under the control of a small molecule delivered to the PACE lagoon at a rate selected by the experimenter.

[1240] In various embodiments, the PEgRNA components would contain a spacer directing the PE to the site of interest on the selection phage and would be designed such that a multiple of three nucleotides could be inserted at the target site such that a new PEgRNA binding site would be introduced, enabling the iterative insertion of one or more codons at the targeted site.

[1241] In parallel, another host *E. coli* strain might include PEgRNAs that would template the removal of one or more codons, enabling loop size to shrink during the evolution. A PACE experiment might utilize a mixture of both strains or alternate the two to permit the slow and controlled addition or removal of loop sequences.

[1242] It is noted that this technique can also be applied to the evolution of antibodies. The binding principles governing antibodies are very similar to those governing monobodies: the

length of antibody complement-determining region loops is critical to their binding function. Further, longer loop lengths have been found to be critical in the development of rare antibodies with broadly protective activity against HIV-1 and other viral infections[9]. Application of PE as described above to an antibody or antibody-derived molecule would permit the generation of antibodies with diverse loop length and varied loop sequence. In combination with PACE, such an approach would permit enhanced binding through loop geometries not accessible to standard PACE, and thus permit evolution of highly functional antibodies.

[1243] Experiments will show the ability to use PE to correct a deleterious mutation in bacteriophage M3 in phage-assisted non-continuous evolution (PANCE), a necessary first step for using PE in continuous evolution (see FIG. 69).

[1244] References for Example 7

[1245] The following references are incorporated herein by reference in their entireties.

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EXAMPLE 8 – IMMUNOEPITOPE INSERTION BY PE

[1246] Precise genome targeting technologies using the CRISPR/Cas system have recently been explored in a wide range of applications, including the insertion of engineered DNA sequences into targeted genomic loci. Previously, homology-directed repair (HDR) has been used for this application, requiring an ssDNA donor template and repair initiation by means of a double-stranded DNA break (DSB). This strategy offers the broadest range of possible changes to be made in cells and is the only method available to insert large DNA sequences into mammalian cells. However, HDR is hampered by undesired cellular side effects stemming from its initiating DSB, such as high levels of indel formation, DNA translocations, large deletions, and P53 activation. In addition to these drawbacks, HDR is

limited by low efficiency in many cell types (T cells are a notable exception to this observation). Recent efforts to overcome these drawbacks include fusing human Rad51 mutants to a Cas9 D10A nickase (RDN), resulting in a DSB-free HDR system that features improved HDR product:indel ratios and lower off target editing, but is still hampered by cell-type dependencies and only modest HDR editing efficiency.

[1247] Recently developed fusions of Cas9 to reverse transcriptases ("Prime editors") coupled with PEgRNAs represent a novel genome editing technology that offers a number of advantages over existing genome editing methods, including the ability to install any single nucleotide substitution, and to insert or delete any short stretch of nucleotides (up to at least several dozen bases) in a site-specific manner. Notably, PE edits are achieved with generally low rates of unintended indels. As such, PE enables targeted insertion-based editing applications that have been previously impossible or impractical.

[1248] This particular invention describes a method for using prime editing as a means to insert known immunogenicity epitopes into endogenous or foreign genomic DNA, resulting in modification of the corresponding proteins for therapeutic or biotechnological applications (see FIGs. 31 and 32). Prior to the invention of prime editing, such insertions could be achieved only inefficiently and with high rates of indel formation from DSBs. prime editing solves the problem of high indel formation from insertion edits while generally offering higher efficiency than HDR. This lower rate of indel formation presents a major advantage of prime editing over HDR as a method for targeted DNA insertions, especially in the described application of inserting immunogenicity epitopes. The length of epitopes is in a range from few bases to hundreds of bases. Prime editor is the most efficient and cleanest technology to achieve such targeted insertions in mammalian cells.

[1249] The key concept of the invention is the use of prime editors to insert a nucleotide sequence containing previously described immunogenicity epitopes into endogenous or foreign genomic DNA for the downregulation and/or destruction of their protein products and/or expressing cell types. Nucleotide sequences for immunogenic epitope insertion would be targeted to genes in a manner to produce fusion proteins of the targeted gene's coded protein and the inserted immunogenic epitope's corresponding protein translation. Patient's immune systems will have been previously trained to recognize these epitopes as a result of standard prior immunization from routine vaccination against, for example, tetanus or diphtheria or measles. As a result of the immunogenic nature of the fused epitopes, patient's

immune systems would be expected to recognize and disable the prime edited protein (not just the inserted epitope) and potentially the cells from which it was expressed.

[1250] Fusions to targeted genes would be engineered as needed to ensure the inserted epitope protein translation is exposed for immune system recognition. This could include targeted nucleotide insertions resulting in protein translations yielding C-terminal fusions of immunogenicity epitopes to targeted genes, N-terminal fusions of immunogenicity epitopes to targeted genes, or the insertion of nucleotides into genes so that immunogenicity epitopes are coded within surfaced-exposed regions of protein structure.

[1251] Protein linkers encoded as nucleotides inserted between the target gene sequence and the inserted immunogenicity epitope nucleotide sequence may need to be engineered as part of this invention to facilitate immune system recognition, cellular trafficking, protein function, or protein folding of the targeted gene. These inserted nucleotide-encoded protein linkers may include (but are not limited to) variable lengths and sequences of the XTEN linker or variable lengths and sequences of Glycine-Serine linkers. These engineered linkers have been previous used to successfully facilitate protein fusions.

[1252] Distinguishing features of this invention include the ability to use previously acquired immune responses to specific amino acid sequences as a means to induce an immune response against otherwise non-immunogenic proteins. Another distinguishing feature is the ability to insert the nucleotide sequences of these immunogenic epitopes in a targets manner that does not induce high levels of unwanted indels as a by-product editing and is efficient in its insertion. The invention discussed herein also has the ability to combine cell type-specific delivery methods (such as AAV serotypes) to insert epitopes in cell types that are of interest to trigger an immune response to.

[1253] Prime editing as a means of inserting immunogenic epitopes into pathogenic genes could be used to program the patient's immune system to fight a wide variety of diseases (not limited to cancer as with immuno-oncology strategies). An immediately relevant use of this technology would be as a cancer therapeutic as it could undermine a tumor's immune escape mechanism by causing an immune response to a relevant oncogene like HER2 or growth factors like EGFR. Such an approach could seem similar to T-cell engineering, but one novel advance of this approach is that it can be utilized in many cell types and for diseases beyond cancer, without needed to generate and introduce engineered T-cells into patients.

[1254] Using PE to insert an immunogenicity epitope which most people are already vaccinated against (tetanus, pertussis, diphtheria, measles, mumps, rubella, etc.) into a foreign

or endogenous gene that drives a disease, so the patient's immune system learns to disable that protein.

[1255] Diseases that stand to have a potential therapeutic benefit from the aforementioned strategy include those caused by aggregation of toxic proteins, such as in fatal familial insomnia. Other diseases that could benefit include those caused by pathogenic overexpression of an otherwise nontoxic endogenous protein, and those caused by foreign pathogens.

[1256] Primary therapeutic indications include those mentioned above such as therapeutics for cancer, prion and other neurodegenerative diseases, infectious diseases, and preventative medicine. Secondary therapeutic indications may include preventative care for patients with late-onset genetic diseases. It is expected that current standard of care medicines may be used in conjunction with prime editing for some diseases, like particularly aggressive cancers, or in cases where medications help alleviate disease symptoms until the disease completely cured.

Below are examples of immunogenic epitopes that can by inserted by prime editing can be used to achieve:

Vaccine	Disease	Epitope Amino Acid Sequence	Example Nucleic Acid Sequence (8)
1	Tetanus toxoid	QYIKANSKFIGITEL (SEQ ID NO: 396)	CATGATATAAAAGCAAATTCTAAATTTATAGGTATAACTGAACTA (SEQ ID NO: 397)
2	Diphtheria toxin mutant CRM197	GADDVVDSSKSF VMENFSSYHGTK PGYVDSIQKGIQK PKSGTQGNYDDD WKEFYSTDNKYD AAGYSVDNENPL SGKAGGVVKVTY PGLTKVLALKVD NAETIKKELGLSL TEPLMEQVGTEEF IKRFGDGASRVVL SLPFAEGSSSVEYI NNWEQAKALSVE LEINFETRGKRGQ DAMYEYMAQAC AGNRVRRSVGSS LSCINLDWDVIRD KTKTKIESLKEHG PIKNKMSESPNKT VSEEKAKQYLEEF HQTALEHPELSEL KTVTGTNPVFAG ANYAAWAVNVA QVIDSETADNLEK TTAALSILPGIGSV MGIADGAVHHNT EEIVAQSIALSSL MVAQAIPLVGEL VDIGFAAYNFVES IINLFQVVHNSYN RPAYSPGHKTQPF LHDGYAVSWNTV EDSIIRTGFQGESG HDIKITAENTPLPI AGVLLPTIPGKLD VNKSKTHISVNGR KIRMRCRAIDGD VTFCRPKSPVYVG NGVHANLHVAFH	GGCGCCGACGACGTGGTGGACAGCA GCAAGAGCTTCGTGATGGAGAACTT CAGCAGCTACCACGGCACCAAGCCC GGCTACGTGGACAGCATCCAGAAGG GCATCCAGAAGCCCAAGAGCGGCAC CCAGGGCAACTACGACGACGACTGG AAGGAGTTCTACAGCACCGACAACA AGTACGACGCCGCCGGCTACAGCGT GGACAACGAGAACCCCCTGAGCGGC AAGGCCGGCGGCGTGGTGAAGGTGA CCTACCCCGGCCTGACCAAGGTGCT GGCCCTGAAGGTGGACAACGCCGAG ACCATCAAGAAGGAGCTGGGCCTGA GCCTGACCGAGCCCCTGATGGAGCA GGTGGGCACCGAGGAGTTCATCAAG AGGTTCGGCGACGGCGCCAGCAGGG TGGTGCTGAGCCTGCCCTTCGCCGA GGGCAGCAGCAGCGTGGAGTACATC AACAACTGGGAGCAGGCCAAGGCCC TGAGCGTGGAGCTGGAGATCAACTT CGAGACCAGGGGCAAGAGGGGCCA GGACGCCATGTACGAGTACATGGCC CAGGCCTGCGCCGGCAACAGGGTGA GGAGGAGCGTGGGCAGCAGCCTGA GCTGCATCAACCTGGACTGGGACGT GATCAGGGACAAGACCAAGACCAA GATCGAGAGCCTGAAGGAGCACGGC CCCATCAAGAACAAGATGAGCGAGA GCCCCAACAAGACCGTGAGCGAGGA GAAGGCCAAGCAGTACCTGGAGGA GTTCCACCAGACCGCCCTGGAGCAC CCCGAGCTGAGCGAGCTGAAGACCG TGACCGGCACCAACCCCGTGTTCGC CGGCGCCAACTACGCCGCCTGGGCC GTGAACGTGGCCCAGGTGATCGACA GCGAGACCGCCGACAACCTGGAGAA GACCACCGCCGCCCTGAGCATCCTG CCCGGCATCGGCAGCGTGATGGGCA TCGCCGACGGCGCCGTGCACCACAA

		RSSSEKIHSNEISS DSIGVLGYQKTV DHTKVNSKLSLFF EIKS (SEQ ID NO: 630)	CACCGAGGAGATCGTGGCCCAGAGC ATCGCCCTGAGCAGCCTGATGGTGG CCCAGGCCATCCCCCTGGTGGGCGA GCTGGTGGACATCGGCTTCGCCGCC TACAACTTCGTGGAGAGCATCATCA ACCTGTTCCAGGTGGTGCACAACAG CTACAACAGGCCCGCCTACAGCCCC GGCCACAAGACCCAGCCCTTCCTGC ACGACGGCTACGCCGTGAGCTGGAA CACCGTGGAGGACAGCATCATCAGG ACCGGCTTCCAGGGCGAGAGCGGCC ACGACATCAAGATCACCGCCGAGAA CACCCCCCTGCCCATCGCCGGCGTG CTGCTGCCCACCATCCCCGGCAAGC TGGACGTGAACAAGAGCAAGACCCA CATCAGCGTGAACGGCAGGAAGATC AGGATGAGGTGCAGGGCCATCGACG GCGACGTGACCTTCTGCAGGCCCAA GAGCCCCGTGTACGTGGGCAACGGC GTGCACGCCAACCTGCACGTGGCCT TCCACAGGAGCAGCAGCGAGAAGAT CCACAGCAACGAGATCAGCAGCGAC AGCATCGGCGTGCTGGGCTACCAGA AGACCGTGGACCACACCAAGGTGAA CAGCAAGCTGAGCCTGTTCTTCGAG ATCAAGAGC (SEQ ID NO: 399)
3	mumps	GTYRLIPNARANL TA (SEQ ID NO: 400)	GGCACCTACAGGCTGATCCCCAACG CCAGGGCCAACCTGACCGCC (SEQ ID NO: 401)
4	mumps	PSKFFTISDSATFA PGPVSNA (SEQ ID NO: 402) PSKLFIMLDNATF APGPVVNA (SEQ ID NO: 404)	Ccgagcaaatttttttaccattagcgatagcgcgacctttgcg ccgggcccggtgagcaacgcg (SEQ ID NO: 403) Ccgagcaaactgtttattatgctggataacgcgacctttgcg ccgggcccggtggtgaacgcg (SEQ ID NO: 405) Selected examples from Hemagglutinin–neuraminidase (HN) diversity among outbreak strains (table1) Divergence between vaccine strain JL5 and outbreak strains (table2)
5	Rubella virus (RV)	TPPPYQVSCGGES DRASARVIDPAA QS (SEQ ID NO: 406)	ACCCCCCCCCCCTACCAGGTGAGCT GCGGCGGCGAGAGCGACAGGGCCA GCGCCAGGGTGATCGACCCCGCCGC CCAGAGC (SEQ ID NO: 407)

6	Hemagglutinin	PEYAYKIVKNKK MEDGFLQGMVD GWYGHHSNEQGS GLMENERTLDKA NPNNDLCSWSDH EASSNNTNQEDLL QRESRRKKRIGTS TLNQRGNCNTKC QTEEARLKREEVS LVKSDQCSNGSL QCRANNSTEQVD (SEQ ID NO: 408)	CCCGAGTACGCCTACAAGATCGTGA AGAACAAGAAGATGGAGGACGGCT TCCTGCAGGGCATGGTGGACGGCTG GTACGGCCACCACAGCAACGAGCAG GGCAGCGGCCTGATGGAGAACGAG AGGACCCTGGACAAGGCCAACCCCA ACAACGACCTGTGCAGCTGGAGCGA CCACGAGGCCAGCAGCAACAACACC AACCAGGAGGACCTGCTGCAGAGGG AGAGCAGGAGGAAGAAGAGGATCG GCACCAGCACCCTGAACCAGAGGGG CAACTGCAACACCAAGTGCCAGACC GAGGAGGCCAGGCTGAAGAGGGAG GAGGTGAGCCTGGTGAAGAGCGACC AGTGCAGCAACGGCAGCCTGCAGTG CAGGGCCAACAACAGCACCGAGCA GGTGGAC (SEQ ID NO: 409)
7	Neuraminidase	TKSTNSRSGGISG PDNEAPVGEAPSP YGDNPRPNDGNN IRIGSKGYNGIITD TIEESCSCYPDAK VVKSVELDSTIWT SGSSPNQKIITIGW DPNGWTGTPMSP NGAYGTDGPSNG QANQHQAESISA GNSSLCPIRDNWH GSNRSWSWPDGA E (SEQ ID NO: 410)	ACCAAGAGCACCAACAGCAGGAGC GGCGGCATCAGCGGCCCCGACAACG AGGCCCCCGTGGGCGAGGCCCCCAG CCCCTACGGCGACAACCCCAGGCCC AACGACGGCAACAACATCAGGATCG GCAGCAAGGGCTACAACGGCATCAT CACCGACACCATCGAGGAGAGCTGC AGCTGCTACCCCGACGCCAAGGTGG TGAAGAGCGTGGAGCTGGACAGCAC CATCTGGACCAGCGGCAGCAGCCCC AACCAGAAGATCATCACCATCGGCT GGGACCCCAACGGCTGGACCGGCAC CCCCATGAGCCCCAACGGCGCCTAC GGCACCGACGGCCCCAGCAACGGCC AGGCCAACCAGCACCAGGCCGAGA GCATCAGCGCCGGCAACAGCAGCCT GTGCCCCATCAGGGACAACTGGCAC GGCAGCAACAGGAGCTGGAGCTGGC CCGACGGCGCCGAG (SEQ ID NO: 411)
8	TAP (transport antigen presentation) on H5N1 virus hemagglutinin	EKIVLLLAMMEKI VLLLAKCQTPMG AIKAVDGVTNKC PYLGSPSF (SEQ ID NO: 412)	GAGAAGATCGTGCTGCTGCTGGCCA TGATGGAGAAGATCGTGCTGCTGCT GGCCAAGTGCCAGACCCCCATGGGC GCCATCAAGGCCGTGGACGGCGTGA CCAACAAGTGCCCCTACCTGGGCAG CCCCAGCTTC (SEQ ID NO: 413)
9	TAP (transport	IRPCFWVELNPNQ KIITIRPCFWVELI	ATCAGGCCCTGCTTCTGGGTGGAGC TGAACCCCAACCAGAAGATCATCAC

	antigen presentation) on h5n1 virus neuraminidase	CYPDAGEIT (SEQ ID NO: 414)	CATCAGGCCCTGCTTCTGGGTGGAG CTGATCTGCTACCCCGACGCCGGCG AGATCACC (SEQ ID NO: 415)
10	hemagglutinin epitopes toward class I HLA	MEKIVLLLAEKIV LLLAMCPYLGSPS FKCQTPMGAIKA VDGVTNK (SEQ ID NO: 416)	ATGGAGAAGATCGTGCTGCTGCTGG CCGAGAAGATCGTGCTGCTGCTGGC CATGTGCCCCTACCTGGGCAGCCCC AGCTTCAAGTGCCAGACCCCCATGG GCGCCATCAAGGCCGTGGACGGCGT GACCAACAAG (SEQ ID NO: 417)
11	neuraminidase epitopes toward class I HLA	NPNQKIITICYPDAG EITIRPCFWVELRPC FWVELI (SEQ ID NO: 418)	AACCCCAACCAGAAGATCATCACCAT CTGCTACCCCGACGCCGGCGAGATCA CCATCAGGCCCTGCTTCTGGGTGGAG CTGAGGCCCTGCTTCTGGGTGGAGCT GATC (SEQ ID NO: 419)
12	hemagglutinin epitopes toward class II HLA	MVSLVKSDQIGTST LNQR (SEQ ID NO: 420)	ATGGTGAGCCTGGTGAAGAGCGACCA GATCGGCACCAGCACCCTGAACCAGA GG (SEQ ID NO: 421)
13	neuraminidase epitopes toward class II HLA	YNGIITDTI (SEQ ID NO: 422)	TACAACGGCATCATCACCGACACCAT C (SEQ ID NO: 423)
14	hemagglutinin epitope H5N1-bound class I and class II HLA	MEKIVLLLAEKIVL LLAMMVSLVKSDQ CPYLGSPSFIGTSTL NQRKCQTPMGAIK AVDGVTNK (SEQ ID NO: 424)	ATGGAGAAGATCGTGCTGCTGCTGGC CGAGAAGATCGTGCTGCTGCTGGCCA TGATGGTGAGCCTGGTGAAGAGCGAC CAGTGCCCCTACCTGGGCAGCCCCAG CTTCATCGGCACCAGCACCCTGAACC AGAGG (SEQ ID NO: 425)
15	neuraminidase epitope H5N1-bound class I and class II HLA	NPNQKIITIYNGIIT DTICYPDAGEITIRP CFWVELRPCFWVE LI (SEQ ID NO: 426)	AACCCCAACCAGAAGATCATCACCAT CTACAACGGCATCATCACCGACACCA TCTGCTACCCCGACGCCGGCGAGATC ACCATCAGGCCCTGCTTCTGGGTGGA GCTGAGGCCCTGCTTCTGGGTGGAGC TGATC (SEQ ID NO: 427)

[1257] Below are additional examples of epitopes that may be integrated into a target gene for immunoepitope taggin:

References cited in Example 8

[1258] The following references are incorporated by reference in their entireties.

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EXAMPLE 9 – *IN VIVO* DELIVERY OF PE AGENTS

[1259] Precise genome targeting technologies using the CRISPR/Cas9 system have recently been explored in a wide range of applications, including gene therapy. A major limitation to the application of Cas9 and Cas9-based genome-editing agents in gene therapy is the size of Cas9 (>4 kb), impeding its efficient delivery via recombinant adeno-associated virus (rAAV). Recently-developed fusions of Cas9 to reverse transcriptases ("Prime editors") represent a novel genome editing technology that possesses a number of advantages over existing

genome editing methods, including the ability to install any single nucleotide substitution, and to insert or delete any arbitrarily-defined short (<~20) stretch of nucleotides in a site-specific manner. As such, this method enables editing of human pathogenic variants that have been intractable to correction previously. The delivery of prime editing reagents could enable correction of genetic sequences that cause human disease, or allow for the installation of disease-preventing gene variants.

[1260] This invention describes methods for delivering prime editors into cells *in vitro* and *in vivo*. Prime editors have been developed and characterized solely in cultured cells. No known method can deliver prime editors *in vivo*. The presently disclosed methods for delivering prime editors via rAAV or pre-assembled ribonucleoprotein (RNP) complexes will overcome several barriers to *in vivo* delivery. For example, the DNA encoding prime editors is larger than the rAAV packaging limit, and so requires special solutions. One such solution is formulating the editor fused to split intein pairs that are packaged into two separate rAAV particles that, when co-delivered to a cell, reconstitute the functional editor protein. Several other special considerations to account for the unique features of prime editing are described, including the optimization of second-site nicking targets and properly packaging prime editors into virus vectors, including lentiviruses and rAAV.

[1261] Distinguishing features include using ribonucleoprotein (RNP) delivery formulations, prime editors and nearby nicking targets can be pre-complexed with their specific sgRNA / PEgRNA. This will enhance the range of possible targetable sites and allow for greater optimization of editing efficiency relative to current data that has used DNA delivery. Using either RNP or mRNA delivery formulations, variant Cas proteins can be used that each complex with their own guide RNA variant. This will also allow for a greater diversity of potential nicking loci, so it is expected that optimization can be achieved for greater efficiency in any given application. Using RNP, it is expected to increase editing specificity base on previous RNP reports (Rees et al., 2017). This would reduce off-target prime editing. Potential architectures for splitting prime editors into two AAV vectors for delivery *in vivo* or *ex vivo* are described. Packaging prime editor into a dual AAV system requires optimization of design considerations including split sites, reconstitution methods (such as inteins), and guide expression architecture. Using a mixture of virus and RNP for delivery of prime editor, it is expected that editing will be controlled over time since RNP eventually degrades *in vivo* which will stop prime editing after RNP is no longer supplied.

[1262] Prime editor ribonucleoprotein (RNP), mRNA with prime editor guide RNA, or DNA can be packaged into lipid nanoparticles, rAAV, or lentivirus and injected, ingested, or inhaled to alter genomic DNA *in vivo* and *ex vivo*, including for the purposes of establishing animal models of human disease, testing therapeutic and scientific hypotheses in animal models of human disease, and treating disease in humans.

[1263] Prime editors could feasibly be used to correct a large fraction of all genetic diseases (~89% of pathogenic human genetic variants in Clinvar), if suitable means of delivery into relevant cell types *in vivo* are developed. Blood diseases, retinal diseases, and liver diseases are the most likely first applications due to established delivery systems for other reagents. AAV capsids, other evolved or engineered viral vectors, and lipid nanoparticle formulations would need to be used in combination with this invention.

[1264] In certain embodiments, one or more of the prime editor domains (e.g., the napDNAbp domain or the RT domain) could be engineered with an intein sequence.

EXAMPLE 10 – USE OF PE TO IDENTIFY OFF-TARGET EDITING

[1265] There are currently no described methods to detect off-target editing with prime editors (prime editing itself has not been published yet). These methods would allow a researcher to identify potential sites of off-target editing using prime editors, which would be important considerations were this technique used to treat genetic disease in patients.

[1266] Methods described here could also be useful to identify off-targets of Cas nucleases. These off-targets have previously been identified using BLESS, Guide-Seq, CIRCLE-Seq, and Digenome-Seq. However, this method is advantageous in the sensitivity and simplicity of the process.

[1267] The key concept of this aspect is the idea of using prime editing to insert an adapter sequence or primer binding site, templated from a PEgRNA, to enable the rapid identification of genomic off-target modification sites of Cas nucleases or prime editors.

[1268] No method to identify in an unbiased manner prime editing off-target sites is known. This method is distinguished from other techniques that identify nuclease off-target sites because the adapter sequence is inserted in the same event as DNA binding and nicking, simplifying the downstream processing.

[1269] The present invention includes identification of off-target editing sites when editing inside a living cell, in tissue culture or animal models (see FIG. 33). To conduct this method, a PEgRNA is generated that has an identical protospacer to the final desired editor (and, if

looking at prime editing off-targets, an identical primer-binding site sequence to the final desired editor), but includes the necessary sequences to install an adapter or primer binding site after reverse transcription by prime editing. *In vivo* editing is conducted using a prime editor or RT-fused nuclease, and isolate genomic DNA. The genomic DNA is fragmented by enzymatic or mechanical means and append a different adapter to sites of DNA fragmentation. PCR is used to amplify from one adapter to the adapter installed via PEgRNA. The resulting product is deep-sequenced to identify all modified sites.

[1270] The invention also includes identification of off-target editing sites using *in vitro* modification of genomic DNA (see FIG. 33). To conduct this method, RNP of purified prime editor protein and a PEgRNA is assembled that will install an adapter or primer binding sequence, but is otherwise the same as the PEgRNA of interest. This RNP is incubated with extracted genomic DNA before or after fragmentation of the DNA and attachment of different adapters to sites of DNA breaks. PCR is used to amplify from fragmented site to the adapter that was installed with PE. Deep sequence to identify sites of modification. This *in vitro* editing method should enhance the sensitivity of detection, because cellular DNA repair will never eliminate the reverse-transcribed DNA adapter added by the prime editor.

[1271] These methods could be used to identify off-target editing for any prime editor, or any genome editor that uses a guideRNA to recognize a target cut site (most Cas nucleases).

[1272] These methods could be applied to all genetic diseases for which genome editors are considered for use in treatment.

EXAMPLE 11 – USE OF PE TO ENABLE CHEMICAL-INDUCED DIMERIZATION OF TARGET PROTEINS *IN VIVO*

[1273] The prime editors described herein may also be used to place dimerization-induced biological processes, such as receptor signaling, under control of a convenient small-molecule drug by the genomic integration of genes encoding small-molecule binding proteins with prime editing is described herein. Using the prime editors described herein, the gene sequence encoding a small-molecule binding protein may be inserted within a gene encoding a target protein of interest in a living cell or patient. This edit alone should have no physiological effect. Upon administration of the small-molecule drug, which typically is a dimeric small molecule that can simultaneously bind to two drug-binding protein domains each of which is fused to a copy of the target protein, the small-molecule induces

dimerization of the targeted protein. This target protein dimerization event then induces a biological signaling event, such as erythropoiesis or insulin signaling.

EXAMPLE 12 - PRIME EDITING: HIGHLY VERSATILE AND PRECISE SEARCH-AND-REPLACE GENOME EDITING IN HUMAN CELLS WITHOUT DOUBLE-STRANDED DNA BREAKS

[1274] Current genome editing methods can disrupt, delete, or insert target genes with accompanying byproducts of double-stranded DNA breaks using programmable nucleases, and install the four transition point mutations at target loci using base editors. Small insertions, small deletions, and the eight transversion point mutations, however, collectively represent most pathogenic genetic variants but cannot be corrected efficiently and without an excess of byproducts in most cell types. Described herein is prime editing, a highly versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 fused to an engineered reverse transcriptase, programmed with an engineered prime editing guide RNA (PEgRNA) that both specifies the target site and encodes the desired edit. Greater than 175 distinct edits in human cells were performed to establish that prime editing can make targeted insertions, deletions, all 12 possible types of point mutations, and combinations thereof efficiently (typically 20-60%, up to 77% in unsorted cells) and with low byproducts (typically 1-10%), without requiring double-stranded breaks or donor DNA templates. Prime editing was applied in human cells to correct the primary genetic causes of sickle cell disease (requiring an A•T-to-T•A transversion in *HBB*) and Tay-Sachs disease (requiring a 4-base deletion in *HEXA*), in both cases efficiently reverting the pathogenic genomic alleles to wild-type with minimal byproducts. Prime editing was also used to create human cell lines with these pathogenic *HBB* transversion and *HEXA* insertion mutations, to install the G127V mutation in *PRNP* that confers resistance to prion disease (requiring a G•C-to-T•A transversion), and to efficiently insert a His6 tag, a FLAG epitope tag, and an extended *LoxP* site into target loci in human cells. Prime editing offers efficiency and product purity advantages over HDR, and complementary strengths and weaknesses compared to base editing. Consistent with its search-and-replace mechanism, which requires three distinct base-pairing events, prime editing is much less prone to off-target DNA modification at known Cas9 off-target sites than Cas9. Prime editing substantially expands the scope and capabilities of genome editing, and in principle can correct ~89% of known pathogenic human genetic variants.

[1275] The ability to make virtually any targeted change in the genome of any living cell or organism is a longstanding aspiration of the life sciences. Despite rapid advances in genome editing technologies, the majority of the >75,000 known human genetic variants associated with diseases[111] cannot be corrected or installed in most therapeutically relevant cells (FIG. 38A). Programmable nucleases such as CRISPR-Cas9 make double-stranded DNA breaks (DSBs) that can disrupt genes by inducing mixtures of insertions and deletions (indels) at target sites[112-114]. Nucleases can also be used to delete target genes[115,116], or insert exogenous genes[117-119], through homology-independent processes. Double- stranded DNA breaks, however, are also associated with undesired outcomes including complex mixtures of products, translocations[120], and p53 activation[121,122]. Moreover, the vast majority of pathogenic alleles differ from their non-pathogenic counterparts by small insertions, deletions, or base substitutions that require much more precise editing technologies to correct (FIG. 38A). Homology-directed repair (HDR) stimulated by nuclease-induced DSBs[123] has been widely used to install a variety of precise DNA changes. HDR, however, relies on exogenous donor DNA repair templates, typically generates an excess of indel byproducts from end-joining repair of DSBs, and is inefficient in most therapeutically relevant cell types (T cells and some stem cells being important exceptions)[124,125]. While enhancing the efficiency and precision of DSB-mediated genome editing remains the focus of promising efforts[126-130], these challenges necessitate the exploration of alternative precision genome editing strategies.

[1276] Base editing can efficiently install or correct the four types of transition mutations (C to T, G to A, A to G, and T to C) without requiring DSBs in a wide variety of cell types and organisms, including mammals[128–131], but cannot currently achieve any of the eight transversion mutations (C to A, C to G, G to C, G to T, A to C, A to T, T to A, and T to G), such as the T•A-to-A•T mutation needed to directly correct the most common cause of sickle cell disease (*HBB* E6V)[132]. In addition, no DSB-free method has been reported to perform target deletions, such as the removal of the 4-base duplication that causes Tay-Sachs disease (*HEXA* 1278+TATC)[133], or targeted insertions, such as the precise 3-base insertion required to directly correct the most common cause of cystic fibrosis (*CFTR* ΔF508)[134]. Targeted transversion point mutations, insertions, and deletions thus are difficult to install or correct efficiently and without excess byproducts in most cell types, even though they collectively account for most known pathogenic alleles (FIG. 38A).

[1277] Described herein is the development of prime editing, a new "search-and-replace" genome editing technology that mediates targeted insertions, deletions, and all 12 possible base-to-base conversions at targeted loci in human cells without requiring double-stranded DNA breaks, or donor DNA templates. Prime editors, initially exemplified by PE1, use a reverse transcriptase fused to a programmable nickase and a prime editing extended guide RNA (PEgRNA) to directly copy genetic information from the extension on the PEgRNA into the target genomic locus. A second-generation prime editor (PE2) uses an engineered reverse transcriptase to substantially increase editing efficiencies with minimal (typically <2%) indel formation, while a third-generation PE3 system adds a second guide RNA to nick the non-edited strand, thereby favoring replacement of the non-edited strand and further increasing editing efficiency, typically, to about 20-50% in human cells with about 1-10% indel formation. PE3 offers far fewer byproducts and higher or similar efficiency compared to optimized Cas9 nuclease-initiated HDR, and offers complementary strengths and weaknesses compared to current-generation base editors.

[1278] PE3 was applied at genomic loci in human HEK293T cells to achieve efficient conversion of *HBB* E6V to wild-type *HBB*, deletion of the inserted TATC to restore *HEXA 1278+TATC* to wild-type *HEXA*, installation in *PRNP* of the G127V mutation that confers resistance to prion disease[135] (requiring a G•C-to-T•A transversion), and targeted insertion of a His_6 tag (18 bp), FLAG epitope tag (24 bp), and extended *LoxP* site for Cre-mediated recombination (44 bp). Prime editing was also successful in three other human cell lines, as well as in post-mitotic primary mouse cortical neurons, with varying efficiencies. Due to a high degree of flexibility in the distance between the initial nick and location of the edit, prime editing is not substantially constrained by the PAM requirement of Cas9 and in principle can target the vast majority of genomic loci. Off-target prime editing is much rarer than off-target Cas9 editing at known Cas9 off-target loci, likely due to the requirement of three distinct DNA base pairing events in order for productive prime editing to take place. By enabling precise targeted insertions, deletions, and all 12 possible classes of point mutations at a wide variety of genomic loci without the need for DSBs or donor DNA templates, prime editing has the potential to advance the study and correction of many gene variants.

Results

Strategy for transferring information from an extended guide RNA into a target DNA locus

[1279] Cas9 targets DNA using a guide RNA containing a spacer sequence that hybridizes to the target DNA site[112–114,136,137]. The aim was to engineer guide RNAs to both specify the DNA target as in natural CRISPR systems[138,139], and also to contain new genetic information that replaces the corresponding DNA nucleotides at the target locus. The direct transfer of genetic information from an extended guide RNA into a specified DNA site, followed by replacement of the original unedited DNA, in principle could provide a general means of installing targeted DNA sequence changes in living cells, without dependence on DSBs or donor DNA templates. To achieve this direct information transfer, the aim was to use genomic DNA, nicked at the target site to expose a 3′-hydroxyl group, to prime the reverse transcription of the genetic information from an extension on the engineered guide RNA (hereafter referred to as the prime editing guide RNA, or PEgRNA) directly into the target site (FIG. 38A).

[1280] These initial steps of nicking and reverse transcription, which resemble mechanisms used by some natural mobile genetic elements[140], result in a branched intermediate with two redundant single-stranded DNA flaps on one strand: a 5′ flap that contains the unedited DNA sequence, and a 3′ flap that contains the edited sequence copied from the PEgRNA (FIG. 38B). To achieve a successful edit, this branched intermediate must be resolved so that the edited 3′ flap replaces the unedited 5′ flap. While hybridization of the 5′ flap with the unedited strand is likely to be thermodynamically favored since the edited 3′ flap can make fewer base pairs with the unedited strand, 5′ flaps are the preferred substrate for structure-specific endonucleases such as FEN1[141], which excises 5′ flaps generated during lagging-strand DNA synthesis and long-patch base excision repair. It was reasoned that preferential 5′ flap excision and 3′ flap ligation could drive the incorporation of the edited DNA strand, creating heteroduplex DNA containing one edited strand and one unedited strand (FIG. 38B).

[1281] Permanent installation of the edit could arise from subsequent DNA repair that resolves the mismatch between the two DNA strands in a manner that copies the information in the edited strand to the complementary DNA strand (FIG. 38C). Based on a similar strategy developed to maximize the efficiency of DNA base editing[131-133], it was envisioned that nicking the non-edited DNA strand, far enough from the site of the initial nick to minimize double-strand break formation, might bias DNA repair to preferentially replace the non-edited strand.

Validation of prime editing steps in vitro and in yeast cells

[1282] Following cleavage of the PAM-containing DNA strand by the RuvC nuclease domain of Cas9, the PAM-distal fragment of this strand can dissociate from otherwise stable Cas9:sgRNA:DNA complexes[143]. It was hypothesized that the 3′ end of this liberated strand might be sufficiently accessible to prime DNA polymerization. Guide RNA engineering efforts[144–146] and crystal structures of Cas9:sgRNA:DNA complexes[147–149] suggest that the 5′ and 3′ termini of the sgRNA can be extended without abolishing Cas9:sgRNA activity. PEgRNAs were designed by extending sgRNAs to include two critical components: a primer binding site (PBS) that allows the 3′ end of the nicked DNA strand to hybridize to the PEgRNA, and a reverse transcriptase (RT) template containing the desired edit that would be directly copied into the genomic DNA site as the 3′ end of the nicked DNA strand is extended across the RNA template by a polymerase (FIG. 38C).

[1283] These hypotheses were tested *in vitro* using purified S. pyogenes Cas9 protein. A series of PEgRNA candidates were constructed by extending sgRNAs on either terminus with a PBS sequence (5 to 6 nucleotides, nt) and an RT template (7 to 22 nt). It was confirmed that 5′-extended PEgRNAs direct Cas9 binding to target DNA, and that both 5′-extended PEgRNAs and 3′-extended PEgRNAs support Cas9-mediated target nicking *in vitro* and DNA cleavage activities in mammalian cells (FIGs. 44A-44C). These candidate PEgRNA designs were tested using pre-nicked 5′-Cy5-labeled dsDNA substrates, catalytically dead Cas9 (dCas9), and a commercial variant of Moloney murine leukemia virus (M-MLV) reverse transcriptase (FIG. 44D). When all components were present, efficient conversion of the fluorescently labeled DNA strand into longer DNA products with gel mobilities, consistent with reverse transcription along the RT template, (FIG. 38D, FIGs. 44D-44E) was observed. Products of desired length were formed with either 5′-extended or 3′-extended PEgRNAs (FIGs. 38D-38E). Omission of dCas9 led to nick translation products derived from reverse transcriptase-mediated DNA polymerization on the DNA template, with no PEgRNA information transfer (FIG. 38D). No DNA polymerization products were observed when the PEgRNA was replaced by a conventional sgRNA, confirming the necessity of the PBS and RT template components of the PEgRNA (FIG. 38D). These results demonstrate that Cas9-mediated DNA melting exposes a single-stranded R-loop that, if nicked, is competent to prime reverse transcription from either a 5′-extended or 3′-extended PEgRNA.

[1284] Next, non-nicked dsDNA substrates were tested with a Cas9 nickase (H840A mutant) that exclusively nicks the PAM-containing strand[112]. In these reactions, 5′-extended PEgRNAs generated reverse transcription products inefficiently, possibly due to impaired

Cas9 nickase activity (FIG. 44F). However, 3′-extended PEgRNAs enabled robust Cas9 nicking and efficient reverse transcription (FIG. 38E). The use of 3′-extended PEgRNAs generated only a single apparent product, despite the potential, in principle, for reverse transcription to terminate anywhere within the remainder of the PEgRNA. DNA sequencing of the products of reactions with Cas9 nickase, RT, and 3′-extended PEgRNAs revealed that the complete RT template sequence was reverse transcribed into the DNA substrate (FIG. 44G). These experiments established that 3′-extended PEgRNAs can template the reverse transcription of new DNA strands while retaining the ability to direct Cas9 nickase activity.

[1285] To evaluate the eukaryotic cell DNA repair outcomes of 3′ flaps produced by PEgRNA- programmed reverse transcription *in vitro*, DNA nicking and reverse transcription using PEgRNAs, Cas9 nickase, and RT *in vitro* on reporter plasmid substrates were performed, and the reaction products were then transformed into yeast (S. cerevisiae) cells (FIG. 45A). Encouragingly, when plasmids were edited *in vitro* with 3′-extended PEgRNAs encoding a T•A-to-A•T transversion that corrects the premature stop codon, 37% of yeast transformants expressed both GFP and mCherry proteins (FIG. 38F, FIG. 45C). Consistent with the results in FIG. 38E and FIG. 44F, editing reactions carried out in vitro with 5′-extended PEgRNAs yielded fewer GFP and mCherry double-positive colonies (9%) than those with 3′-extended PEgRNAs (FIG. 38F and FIG. 45D). Productive editing was also observed using 3′-extended PEgRNAs that insert a single nucleotide (15% double-positive transformants) or delete a single nucleotide (29% double-positive transformants) to correct frameshift mutations (FIG. 38F and FIGs. 45E-45F). DNA sequencing of edited plasmids recovered from double-positive yeast colonies confirmed that the encoded transversion edit occurred at the desired sequence position (FIG. 45G). These results demonstrate that DNA repair in eukaryotic cells can resolve 3′ DNA flaps arising from prime editing to incorporate precise DNA edits including transversions, insertions, and deletions.

Design of prime editor 1 (PE1)

[1286] Encouraged by the results *in vitro* and in yeast, a prime editing system with a minimum number of components capable of editing genomic DNA in mammalian cells was sought for development. It was hypothesized that 3′-extended PEgRNAs (hereafter referred to simply as PEgRNAs, FIG. 39A) and direct fusions of Cas9 H840A to reverse transcriptase via a flexible linker may constitute a functional two-component prime editing system. HEK293T (immortalized human embryonic kidney) cells were transfected with one plasmid

encoding a fusion of wild-type M-MLV reverse transcriptase to either terminus of Cas9 H840A nickase as well as a second plasmid encoding a PEgRNA. Initial attempts led to no detectable T•A-to-A•T conversion at the HEK3 target locus.

[1287] Extension of the PBS in the PEgRNA to 8-15 bases (FIG. 39A), however, led to detectable T•A-to-A•T editing at the HEK3 target site (FIG. 39B), with higher efficiencies for prime editor constructs in which the RT was fused to the C-terminus of Cas9 nickase (3.7% maximal T•A-to-A•T conversion with PBS lengths ranging from 8-15 nt) compared to N-terminal RT–Cas9 nickase fusions (1.3% maximal T•A-to-A•T conversion) (FIG. 39B; all mammalian cell data herein reports values for the entire treated cell population, without selection or sorting, unless otherwise specified). These results suggest that wild-type M-MLV RT fused to Cas9 requires longer PBS sequences for genome editing in human cells compared to what is required *in vitro* using the commercial variant of M-MLV RT supplied in trans. This first- generation wild-type M-MLV reverse transcriptase fused to the C-terminus of Cas9 H840A nickase was designated as PE1.

[1288] The ability of PE1 to precisely introduce transversion point mutations at four additional genomic target sites specified by the PEgRNA (FIG. 39C) was tested. Similar to editing at the HEK3 locus, efficiency at these genomic sites was dependent on PBS length, with maximal editing efficiencies ranging from 0.7-5.5% (FIG. 39C). Indels from PE1 were low, averaging 0.2±0.1% for the five sites under conditions that maximized each site's editing efficiency (FIG. 46A). PE1 was also able to install targeted insertions and deletions, exemplified by a single-nucleotide deletion (4.0% efficiency), a single-nucleotide insertion (9.7%), and a three-nucleotide insertion (17%) at the HEK3 locus (FIG. 39C). These results establish the ability of PE1 to directly install targeted transversions, insertions, and deletions without requiring double-stranded DNA breaks or DNA templates.

[1289] Design of prime editor 2 (PE2)

[1290] While PE1 can install a variety of edits at several loci in HEK293T cells, editing efficiencies were generally low (typically ≤5%) (FIG. 39C). It was hypothesized that engineering the reverse transcriptase in PE1 might improve the efficiency of DNA synthesis within the unique conformational constraints of the prime editing complex, resulting in higher genome editing yields. M-MLV RT mutations have been previously reported that increase enzyme thermostability[150,151], processivity[150], and DNA:RNA heteroduplex substrate affinity[152], and that inactivate RNaseH activity[153]. 19 PE1 variants were constructed

containing a variety of reverse transcriptase mutations to evaluate their prime editing efficiency in human cells.

[1291] First, a series of M-MLV RT variants that previously emerged from laboratory evolution for their ability to support reverse transcription at elevated temperatures[150] were investigated. Successive introduction of three of these amino acid substitutions (D200N, L603W, and T330P) into M-MLV RT, hereafter referred to as M3, led to a 6.8-fold average increase in transversion and insertion editing efficiency across five genomic loci in HEK293T cells compared to that of PE1 (FIGs. 47A-47S).

[1292] Next, in combination with M3, additional reverse transcriptase mutations that were previously shown to enhance binding to template:PBS complex, enzyme processivity, and thermostability[152] were tested. Among the 14 additional mutants analyzed, a variant with T306K and W313F substitutions, in addition to the M3 mutations, improved editing efficiency an additional 1.3-fold to 3.0-fold compared to M3 for six transversion or insertion edits across five genomic sites in human cells (FIGs. 47A-47S). This pentamutant of M-MLV reverse transcriptase incorporated into the PE1 architecture (Cas9 H840A–M-MLV RT (D200N L603W T330P T306K W313F)) is hereafter referred to as PE2.

[1293] PE2 installs single-nucleotide transversion, insertion, and deletion mutations with substantially higher efficiency than PE1 (FIG. 39C), and is compatible with shorter PBS PEgRNA sequences (FIG. 39C), consistent with an enhanced ability to productively engage transient genomic DNA:PBS complexes. On average, PE2 led to a 1.6- to 5.1-fold improvement in prime editing point mutation efficiency over PE1 (FIG. 39C), and in some cases dramatically improved editing yields up to 46-fold (FIG. 47F and FIG. 47I). PE2 also effected targeted insertions and deletions more efficiently than PE1, achieving the targeted insertion of the 24-bp FLAG epitope tag at the HEK3 locus with 4.5% efficiency, a 15-fold improvement over the efficiency of installing this insertion with PE1 (FIG. 47D), and mediated a 1-bp deletion in HEK3 with 8.6% efficiency, 2.1-fold higher than that of PE1 (FIG. 39C). These results establish PE2 as a more efficient prime editor than PE1.

[1294] Optimization of PEgRNA features

[1295] The relationship between PEgRNA architecture and prime editing efficiency was systematically probed at five genomic loci in HEK293T cells with PE2 (FIG. 39C). In general, priming sites with lower GC content required longer PBS sequences (EMX1 and RNF2, containing 40% and 30% GC content, respectively, in the first 10 nt upstream of the

nick), whereas those with greater GC content supported prime editing with shorter PBS sequences (HEK4 and FANCF, containing 80% and 60% GC content, respectively, in the first 10 nt upstream of the nick) (FIG. 39C), consistent with the energetic requirements for hybridization of the nicked DNA strand to the PEgRNA PBS. No PBS length or GC content level was strictly predictive of prime editing efficiency, and other factors such as secondary structure in the DNA primer or PEgRNA extension may also influence editing activity. It is recommended to start with a PBS length of ~13 nt for a typical target sequence, and exploring different PBS lengths if the sequence deviates from ~40-60% GC content. When necessary, optimal PBS sequences should be determined empirically.

[1296] Next, the performance determinants of the RT template portion of the PEgRNA were studied. PEgRNAs with RT templates ranging from 10-20 nt in length were systemically evaluated at five genomic target sites using PE2 (FIG. 39D) and with longer RT templates as long as 31 nt at three genomic sites (FIGs. 48A-48C). As with PBS length, RT template length also could be varied to maximize prime editing efficiency, although in general many RT template lengths ≥10 nt long support more efficient prime editing (FIG. 39D). Since some target sites preferred longer RT templates (>15 nt) to achieve higher editing efficiencies (FANCF, EMX1), while other loci preferred short RT templates (HEK3, HEK4) (FIG. 39D), it is recommend both short and long RT templates be tested when optimizing a PEgRNA, starting with ~10-16 nt.

[1297] Importantly, RT templates that place a C as the nucleotide adjacent to the terminal hairpin of the sgRNA scaffold generally resulted in lower editing efficiency compared to other PEgRNAs with RT templates of similar length (FIGs. 48A-48C). Based on the structure of sgRNAs bound to Cas9[148,149], it was speculated that the presence of a C as the first nucleotide of the 3′ extension of a canonical sgRNA can disrupt the sgRNA scaffold fold by pairing with G81, a nucleotide that natively forms a pi stack with Tyr 1356 in Cas9 and a non-canonical base pair with sgRNA A68. Since many RT template lengths support prime editing, it is recommended to choose PEgRNAs in which the first base of the 3′ extension (the last reverse- transcribed base of the RT template) is not C.

[1298] Design of prime editor 3 systems (PE3 and PE3b)

[1299] While PE2 can transfer genetic information from the PEgRNA to the target locus more efficiently than PE1, the manner in which the cell resolves the resulting heteroduplex DNA created by one edited strand and one unedited strand determines if the edit is durable. A

previous development of base editing faced a similar challenge since the initial product of cytosine or adenine deamination is heteroduplex DNA containing one edited and one non-edited strand. To increase the efficiency of base editing, a Cas9 D10A nickase was used to introduce a nick into the non-edited strand and to direct DNA repair to that strand, using the edited strand as a template[129,130,142]. To exploit this principle to enhance prime editing efficiencies, a similar strategy of nicking the non-edited strand using the Cas9 H840A nickase already present in PE2 and a simple sgRNA to induce preferential replacement of the non-edited strand by the cell (FIG. 40A) was tested. Since the edited DNA strand was also nicked to initiate prime editing, a variety of sgRNA-programmed nick locations were tested on the non-edited strand to minimize the production of double-stranded DNA breaks that lead to indels.

[1300] This PE3 strategy was first tested at five genomic sites in HEK293T cells by screening sgRNAs that induce nicks located 14 to 116 bases from the site of the PEgRNA-induced nick, either 5′ or 3′ of the PAM. In four of the five sites tested, nicking the non-edited strand increased the amount of indel-free prime editing products compared to the PE2 system by 1.5- to 4.2-fold, to as high as 55% (FIG. 40B). While the optimal nicking position varied depending on the genomic site, nicks positioned 3′ of the PAM (positive distances in FIG. 40B) approximately 40-90 bp from the PEgRNA-induced nick generally produced favorable increases in prime editing efficiency (averaging 41%) without excess indel formation (6.8% average indels for the sgRNA resulting in the highest editing efficiency for each of the five sites tested) (FIG. 40B). As expected, at some sites, placement of the non-edited strand nick within 40 bp of the PEgRNA-induced nick led to large increases in indel formation up to 22% (FIG. 40B), presumably due to the formation of a double-strand break from nicking both strands close together. At other sites, however, nicking as close as 14 bp away from the PEgRNA-induced nick produced only 5% indels (FIG. 40B), suggesting that locus-dependent factors control conversion of proximal dual nicks into double-strand DNA breaks. At one tested site (HEK4), complementary strand nicks either provided no benefit or led to indel levels that surpassed editing efficiency (up to 26%), even when placed at distances >70 bp from the PEgRNA-induced nick, consistent with an unusual propensity of the edited strand at that site to be nicked by the cell, or to be ligated inefficiently. It is recommend to start with non-edited strand nicks approximately 50 bp from the PEgRNA-mediated nick, and to test alternative nick locations if indel frequencies exceed acceptable levels.

[1301] This model for how complementary strand nicking improved prime editing efficiency (FIG. 40A) predicted that nicking the non-edited strand only *after* edited strand flap resolution could minimize the presence of concurrent nicks, decreasing the frequency of double-strand breaks that go on to form indels. To achieve temporal control over non-edited strand nicking, sgRNAs with spacer sequences that match the edited strand, but not the original allele, were designed. Using this strategy, referred to hereafter as PE3b, mismatches between the spacer and the unedited allele should disfavor nicking by the sgRNA until after the editing event on the PAM strand takes place. This PE3b approach was tested with five different edits at three genomic sites in HEK293T cells and compared outcomes to those achieved with PE2 and PE3 systems. In all cases, PE3b was associated with substantially lower levels of indels compared to PE3 (3.5- to 30-fold, averaging 12-fold lower indels, or 0.85%), without any evident decrease in overall editing efficiency compared to PE3 (FIG. 40C). Therefore, when the edit lay within a second protospacer, the PE3b system could decrease indels while still improving editing efficiency compared to PE2, often to levels similar to those of PE3 (FIG. 40C).

[1302] Together, these findings established that PE3 systems (Cas9 nickase–optimized reverse transcriptase + PEgRNA + sgRNA) improved editing efficiencies ~3-fold compared with PE2 (FIGs. 40B-40C). PE3 was accompanied by wider ranges of indels than PE2, as expected given the additional nicking activity of PE3. The use of PE3 is recommended when prioritizing prime editing efficiency. When minimization of indels is critical, PE2 offers ~10-fold lower indel frequencies. When it is possible to use a sgRNA that recognizes the installed edit to nick the non-edited strand, the PE3b system can achieve PE3-like editing levels while greatly reducing indel formation.

[1303] To demonstrate the targeting scope and versatility of prime editing with PE3, the installation of all possible single nucleotide substitutions across the +1 to +8 positions (counting the first base 3′ of the PEgRNA-induced nick as position +1) of the HEK3 target site using PE3 and PEgRNAs with 10-nucleotide RT templates (FIG. 41A) was explored. Collectively, these 24 distinct edits cover all four transition mutations and all eight transversion mutations, and proceed with editing efficiencies (containing no indels) averaging 33±7.9% (ranging between 14% and 48%), with an average of 7.5±1.8% indels.

[1304] Importantly, long-distance RT templates could also give rise to efficient prime editing with PE3. For example, using PE3 with a 34-nt RT template, point mutations were installed at positions +12, +14, +17, +20, +23, +24, +26, +30, and +33 (12 to 33 bases from the

PEgRNA- induced nick) in the HEK3 locus with an average of 36±8.7% efficiency and 8.6±2.0% indels (FIG, 41B). Although edits beyond the +10 position at other loci were not attempted, other RT templates ≥30 nt at three alternative sites also support efficient editing (FIGs. 48A-C). The viability of long RT templates enabled efficient prime editing for dozens of nucleotides from the initial nick site. Since an NGG PAM on either DNA strand occurs on average every ~8 bp, far less than maximum distances between the edit and the PAM that support efficient prime editing, prime editing is not substantially constrained by the availability of a nearby PAM sequence, in contrast with other precision genome editing methods[125,142,154]. Given the presumed relationship between RNA secondary structure and prime editing efficiency, when designing PEgRNAs for long-range edits it is prudent to test RT templates of various lengths and, if necessary, sequence compositions (e.g., synonymous codons) to optimize editing efficiency.

[1305] To further test the scope and limitations of the PE3 system for introducing transition and transversion point mutations, 72 additional edits covering all 12 possible types of point mutations across six additional genomic target sites (FIG. 41C-41H) were tested. Overall, indel-free editing efficiency averaged 25±14%, while indel formation averaged 8.3±7.5%. Since the PEgRNA RT template included the PAM sequence, prime editing could induce changes to the PAM sequence. In these cases, higher editing efficiency (averaging 39±9.7%) and lower indel generation (averaging 5.0±2.9%) were observed (FIGs. 41A-41K, point mutations at positions +5 or +6). This increase in efficiency and decrease in indel formation for PAM edits may arise from the inability of the Cas9 nickase to re-bind and nick the edited strand prior to the repair of the complementary strand. Since prime editing supports combination edits with no apparent loss of editing efficiency, editing the PAM, in addition to other desired changes, when possible, is recommended.

[1306] Next, 14 targeted small insertions and 14 targeted small deletions at seven genomic sites using PE3 (FIG. 41I) were performed. Targeted 1-bp insertions proceeded with an average efficiency of 32±9.8%, while 3-bp insertions were installed with an average efficiency of 39±16%. Targeted 1-bp and 3-bp deletions were also efficient, proceeding with an average yield of 29±14% and 32±11%, respectively. Indel generation (beyond the targeted insertion or deletion) averaged 6.8±5.4%. Since insertions and deletions introduced between positions +1 and +6 alter the position or the structure of the PAM, it was speculated that insertion and deletion edits in this range are typically more efficient due to the inability of

Cas9 nickase to re-bind and nick the edited DNA strand prior to repair of the complementary strand, similar to point mutations that edit the PAM.

[1307] PE3 was also tested for its ability to mediate larger precise deletions of 5 bp to 80 bp at the HEK3 site (FIG. 41J). Very high editing efficiencies (52 to 78%) were observed for 5-, 10-, and 15-bp deletions when using a 13-nt PBS and an RT template that contained 29, 24, or 19 bp of homology to the target locus, respectively. Using a 26-nt RT template supported a larger deletion of 25 bp with 72±4.2% efficiency, while a 20-nt RT template enabled an 80-bp deletion with an efficiency of 52±3.8%. These targeted deletions were accompanied by indel frequencies averaging 11±4.8% (FIG. 41J).

[1308] Finally, the ability of PE3 to mediate 12 combinations of multiple edits at the same target locus consisting of insertions and deletions, insertions and point mutations, deletions and point mutations, or two point mutations across three genomic sites was tested. These combination edits were very efficient, averaging 55% of the target edit with 6.4% indels (FIG. 41K), and demonstrating the ability of prime editing to make combinations of precision insertions, deletions, and point mutations at individual target sites with high efficiency and low indel frequencies.

[1309] Together, the examples in FIGs. 41A-41K represent 156 distinct transition, transversion, insertion, deletion, and combination edits across seven human genomic loci. These findings establish the versatility, precision, and targeting flexibility of prime editing.

[1310] Prime editing compared with base editing

[1311] Current-generation cytidine base editors (CBEs) and adenine base editors (ABEs) can install C•G-to-T•A transition mutations and A•T-to-G•C transition mutations with high efficiency and low indels[129,130,142]. The application of base editing can be limited by the presence of multiple cytidine or adenine bases within the base editing activity window (typically ~5-bp wide), which gives rise to unwanted bystander edits[129,130,142,155,] or by the absence of a PAM positioned approximately 15±2 nt from the target nucleotide[142,156]. It was anticipated that prime editing could be particularly useful for precise installation of transitions mutations without bystander edits, or when the lack of suitably positioned PAMs precludes favorable positioning the target nucleotide within the CBE or ABE activity window.

[1312] Prime editing and cytosine base editing was compared by editing three genomic loci that contain multiple target cytidines in the canonical base editing window (protospacer

positions 4-8, counting the PAM as positions 21-23) using optimized CBEs[157] without nickase activity (BE2max) or with nickase activity (BE4max), or using the analogous PE2 and PE3 prime editing systems. Among the nine total target cytosines within the base editing windows of the three sites, BE4max yielded 2.2-fold higher average total C•G-to-T•A conversion than PE3 for bases in the center of the base editing window (protospacer positions 5-7, FIG. 42A). Likewise, non-nicking BE2max outperformed PE2 by 1.4-fold on average at these well-positioned bases (FIG. 42A). However, PE3 outperformed BE4max by 2.7-fold, and PE2 outperformed BE2max by 2.0-fold, for cytosines beyond the center of the base editing window (average editing of 40±17% for PE3 vs. 15±18% for BE4max, and 22±11% for PE2 vs. 11±13% for BE2max). Overall, indel frequencies for PE2 were very low (averaging 0.86±0.47%), and for PE3 were similar to or modestly higher than that of BE4max (BE4max range: 2.5% to 14%; PE3 range: 2.5% to 21%) (FIG. 42B).

[1313] When comparing the efficiency of base editing to prime editing for installation of *precise* C•G-to-T•A edits (without any bystander editing), the efficiency of prime editing greatly exceeded that of base editing at the above sites, which like most genomic DNA sites, contain multiple cytosines within the ~5-bp base editing window (FIG. 42C). At these sites, such as EMX1, which contains cytosines at protospacer positions C5, C6, and C7, BE4max generated few products containing only the single target base pair conversion with no bystander edits. In contrast, prime editing at this site could be used to selectively install a C•G-to-T•A edit at any position or combination of positions (C5, C6, C7, C5+C6, C6+C7, C5+C7, or C5+C6+C7) (FIG. 42C). All precise one-base or two-base edits (that is, edits that do not modify any other nearby bases) were much more efficient with PE3 or PE2 than with BE4max or BE2, respectively, while the three-base C•G-to-T•A edit was more efficient with BE4max (FIG. 42C), reflecting the propensity of base editors to edit all target bases within the activity window. Taken together, these results demonstrate that cytosine base editors can result in higher levels of editing at optimally positioned target bases than PE2 or PE3, but prime editing can outperform base editing at non-optimally positioned target bases, and can edit with much higher precision with multiple editable bases.

[1314] A•T-to-G•C editing was compared at two genomic loci by an optimized non-nicking ABE (ABEmax[152] with a dCas9 instead of a Cas9 nickase, hereafter referred to as ABEdmax) versus PE2, and by the optimized nicking adenine base editor ABEmax versus PE3. At a site that contains two target adenines in the base editing window (HEK3), ABEs were more efficient than PE2 or PE3 for conversion of A5, but PE3 was more efficient for conversion of

A8, which lies at the edge of the ABEmax editing window (FIG. 42D). When comparing the efficiency of precision edits in which only a single adenine is converted, PE3 outperformed ABEmax at both A5 and A8 (FIG. 42E). Overall, ABEs produced far fewer indels at HEK3 than prime editors (0.19±0.02% for ABEdmax vs. 1.5±0.46% for PE2, and 0.53±0.16% for ABEmax vs. 11±2.3% for PE3, FIG. 42F). At FANCF, in which only a single A is present within the base editing window, ABE2 and ABEmax outperformed their prime editing counterparts in total target base pair conversion by 1.8- to 2.9-fold, with virtually all edited products from both base editing and prime editing containing only the precise edit (FIGs. 42D-42E). As with the HEK3 site, ABEs produced far fewer indels at the FANCF site (FIG. 42F).

[1315] Collectively, these results indicate that base editing and prime editing offer complementary strengths and weaknesses for making targeted transition mutations. For cases in which a single target nucleotide is present within the base editing window, or when bystander edits are acceptable, current base editors are typically more efficient and result in fewer indels than prime editors. When multiple cytosines or adenines are present and bystander edits are undesirable, or when target bases are poorly positioned for base editing relative to available PAMs, prime editors offer substantial advantages.

[1316] Off-target prime editing

[1317] To result in productive editing, prime editing requires target locus:PEgRNA spacer complementary for the Cas9 domain to bind, target locus:PEgRNA PBS complementarity for PEgRNA-primed reverse transcription to initiate, and target locus:reverse transcriptase product complementarity for flap resolution. It was hypothesized that these three distinct DNA hybridization requirements may minimize off-target prime editing compared to that of other genome editing methods. To test this possibility, HEK293T cells were treated with PE3 or PE2 and 16 total PEgRNAs designed to target four on-target genomic loci, with Cas9 and the four corresponding sgRNAs targeting the same protospacers, or with Cas9 and the same 16 PEgRNAs. These four target loci were chosen because each has at least four well-characterized off-target sites for which Cas9 and the corresponding on-target sgRNA in HEK293T cells is known to cause substantial off-target DNA modification[118,159]. Following treatment, the four on-target loci and the top four known Cas9 off-target sites for each on-target spacer, were sequenced, for a total of 16 off-target sites (Table 1).

[1318] Consistent with previous studies[118], Cas9 and the four target sgRNAs modified all 16 of the previously reported off-target loci (FIG. 42G). Cas9 off-target modification efficiency among the four off-target sites for the HEK3 target locus averaged 16%. Cas9 and the sgRNA targeting HEK4 resulted in an average of 60% modification of the four tested known off-target sites. Likewise, off-target sites for EMX1 and FANCF were modified by Cas9:sgRNA at an average frequency of 48% and 4.3%, respectively (FIG. 42G). It was noted that PEgRNAs with Cas9 nuclease modified on-target sites at similar (1- to 1.5-fold lower) efficiency on average compared to sgRNAs, while PEgRNAs with Cas9 nuclease modified off-target sites at ~4-fold lower average efficiency than sgRNAs.

[1319] Strikingly, PE3 or PE2 with the same 16 tested PEgRNAs containing these four target spacers resulted in much lower off-target editing (FIG. 42H). Of the 16 sites known to undergo off- target editing by Cas9+sgRNA, PE3+PEgRNAs or PE2+PEgRNAs resulted in detectable off-target prime editing at only 3 of 16 off-target sites, with only 1 of 16 showing off-target editing efficiency ≥1% (FIG. 42H). Average off-target prime editing for the PEgRNAs targeting HEK3, HEK4, EMX1, and FANCF at these 16 known Cas9 off-target sites was <0.1%, <2.2±5.2%, <0.1%, and <0.13±0.11%, respectively (FIG. 42H). Notably, at the HEK4 off-target 3 site that Cas9+PEgRNA1 edits with 97% efficiency, PE2+PEgRNA1 results in only 0.7% off-target editing despite sharing the same spacer sequence, demonstrating how the two additional DNA hybridization events required for prime editing compared to Cas9 editing can greatly reduce off-target editing. Taken together, these results suggest that PE3 and PEgRNAs induce much lower off-target DNA editing in human cells than Cas9 and sgRNAs that target the same protospacers.

[1320] Reverse transcription of 3′-extended PEgRNAs in principle can proceed into the guide RNA scaffold. If the resulting 3′ flap, despite a lack of complementary at its 3′ end with the unedited DNA strand, is incorporated into the target locus, the outcome is insertion of PEgRNA scaffold nucleotides that contributes to indel frequency. We analyzed sequencing data from 66 PE3-mediated editing experiments at four loci in HEK293T cells and observed PEgRNA scaffold insertion at a low frequency, averaging 1.7±1.5% total insertion of any number of PEgRNA scaffold nucleotides (FIGs. 56A-56D). It is speculated that inaccessibility of the guide RNA scaffold to the reverse transcriptase due to Cas9 domain binding, as well as cellular excision during flap resolution of the mismatched 3′ end of the 3′ flap that results from PEgRNA scaffold reverse transcription, minimizes products that incorporate PEgRNA scaffold nucleotides. While such events are rare, future efforts to

engineer PEgRNAs or prime editor proteins that minimize PEgRNA scaffold incorporation may further decrease indel frequencies.

[1321] Deaminases in some base editors can act in a Cas9-independent manner, resulting in low-level but widespread off-target DNA editing among first-generation CBEs (but not ABEs)[160–162] and off-target RNA editing among first-generation CBEs and ABEs[163–165], although newer CBE and ABE variants with engineered deaminases greatly reduce Cas9-independent off-target DNA and RNA editing[163–165]. Prime editors lack base-modification enzymes such as deaminases, and therefore have no inherent ability to modify DNA or RNA bases in a Cas9- independent manner.

[1322] While the reverse transcriptase domain in prime editors in principle could process properly primed RNA or DNA templates in cells, it was noted that retrotransposons such as those in the LINE-1 family[166,] endogenous retroviruses[167,168], and human telomerase all provided active endogenous human reverse transcriptases. Their natural presence in human cells suggests that reverse transcriptase activity itself is not substantially toxic. Indeed, no PE3-dependent differences were observed in HEK293T cell viability compared to that of controls expressing dCas9, Cas9 H840A nickase, or PE2 with R110S+K103L (PE2-dRT) mutations that inactivate the reverse transcriptase[169] (FIGs. 49A-49B).

[1323] The above data and analyses notwithstanding, additional studies are needed to assess off-target prime editing in an unbiased, genome-wide manner, as well as to characterize the extent to which the reverse transcriptase variants in prime editors, or prime editing intermediates, may affect cells.

[1324] Prime editing pathogenic transversion, insertion, and deletion mutations in human cells

[1325] The ability of PE3 to directly install or correct in human cells transversion, small insertion, and small deletion mutations that cause genetic diseases, was tested. Sickle cell disease is most commonly caused by an A•T-to-T•A transversion mutation in HBB, resulting in the mutation of Glu6→Val in beta-globin. Treatment of hematopoietic stem cells *ex vivo* with Cas9 nuclease and a donor DNA template for HDR, followed by enrichment of edited cells, transplantation, and engraftment is a promising potential strategy for the treatment of sickle-cell disease[170]. However, this approach still generates many indel-containing byproducts in addition to the correctly edited HBB allele[170-171]. While base editors generally

produce far fewer indels, they cannot currently make the T•A-to-A•T transversion mutation needed to directly restore the normal sequence of HBB.

[1326] PE3 was used to install the HBB E6V mutation in HEK293T cells with 44% efficiency and 4.8% indels (FIG. 43A. From the mixture of PE3-treated cells, we isolated six HEK293T cell lines that are homozygous (triploid) for the HBB E6V allele (FIGs. 53A-53D), demonstrating the ability of prime editing to generate human cell lines with pathogenic mutations. To correct the HBB E6V allele to wild-type HBB, we treated homozygous HBB E6V HEK293T cells with PE3 and a PEgRNA programmed to directly revert the HBB E6V mutation to wild-type HBB. In total, 14 PEgRNA designs were tested. After three days, DNA sequencing revealed that all 14 PEgRNAs when combined with PE3 gave efficient correction of HBB E6V to wild-type HBB (≥26% wild-type HBB without indels), and indel levels averaging 2.8±0.70% (FIG. 50A). The best PEgRNA resulted in 52% correction of HBB E6V to wild-type with 2.4% indels (FIG. 43A). Introduction of a silent mutation that modifies the PAM recognized by the PEgRNA modestly improved editing efficiency and product purity, to 58% correction with 1.4% indels (FIG. 43A). These results establish that prime editing can install and correct a pathogenic transversion point mutation in a human cell line with high efficiency and minimal byproducts.

[1327] Tay-Sachs disease is most often caused by a 4-bp insertion into the HEXA gene (HEXA 1278+TATC)[136]. PE3 was used to install this 4-bp insertion into HEK293T cells with 31% efficiency and 0.8% indels (FIG. 43B), and isolated two HEK293T cell lines that are homozygous for the HEXA 1278+TATC allele (FIGs. 53A-53D). These cells were used to test 43 PEgRNAs and three nicking sgRNAs with PE3 or PE3b systems for correction of the pathogenic insertion in HEXA (FIG. 50B), either by perfect reversion to the wild-type allele or by a shifted 4-bp deletion that disrupts the PAM and installs a silent mutation. Nineteen of the 43 PEgRNAs tested resulted in ≥20% editing. Perfect correction to wild-type HEXA with PE3 or PE3b and the best PEgRNA proceeded with similar average efficiencies (30% for PE3 vs. 33% for PE3b), but the PE3b system was accompanied by 5.3-fold fewer indel products (1.7% for PE3 vs. 0.32% for PE3b) (FIG. 43B and FIG. 50B). These findings demonstrate the ability of prime editing to make precise small insertions and deletions that install or correct a pathogenic allele in mammalian cells efficiently and with a minimum of byproducts.

[1328] Finally, the installation of a protective SNP into PRNP, the gene encoding the human prion protein (PrP), was tested. PrP misfolding causes progressive and fatal neurodegenerative prion disease that can arise spontaneously, through inherited dominant

mutations in the PRNP gene, or through exposure to misfolded PrP[172]. A naturally occurring PRNP G127V mutant allele confers resistance to prion disease in humans[138] and mice[173]. PE3 was used to install G127V into the human PRNP allele in HEK293T cells, which requires a G•C-to-T•A transversion. Four PEgRNAs and three nicking sgRNAs were evaluated with the PE3 system. After three days of exposure to the most effective PE3 and PEgRNA, DNA sequencing revealed 53±11% efficiency of installing the G127V mutation and indel levels of 1.7±0.7% (FIG. 43C). Taken together, these results establish the ability of prime editing in human cells to install or correct transversion, insertion, or deletion mutations that cause or confer resistance to disease efficiently, and with a minimum of byproducts.

[1329] Prime editing in various human cell lines and primary mouse neurons

[1330] Next, prime editing was tested for its ability to edit endogenous sites in three additional human cell lines. In K562 (leukemic bone marrow) cells, PE3 was used to perform transversion edits in the HEK3, EMX1, and FANCF sites, as well as the 18-bp insertion of a 6xHis tag in HEK3. An average editing efficiency of 15-30% was observed for each of these four PE3-mediated edits, with indels averaging 0.85-2.2% (FIG. 43A). In U2OS (osteosarcoma) cells, transversion mutations in HEK3 and FANCF were installed, as well as a 3-bp insertion and 6xHis tag insertion into HEK3, with 7.9-22% editing efficiency that exceeded indel formation 10- to 76 fold(FIG. 43A). Finally, in HeLa (cervical cancer) cells, a 3-bp insertion into HEK3 was performed, with 12% average efficiency and 1.3% indels (FIG. 43A). Collectively, these data indicate that multiple cell lines beyond HEK293T cells support prime editing, although editing efficiencies vary by cell type and are generally less efficient than in HEK293T cells. Editing:indel ratios remained high in all tested human cell lines.

[1331] To determine if prime editing is possible in post-mitotic, terminally differentiated primary cells, primary cortical neurons harvested from E18.5 mice were transduced with a dual split-PE3 lentiviral delivery system in which split-intein splicing[203] reconstitutes PE2 protein from N-terminal and C-terminal halves, each delivered from a separate virus. To restrict editing to post-mitotic neurons, the human synapsin promoter, which is highly specific for mature neurons[204], was used to drive expression of both PE2 protein components. GFP was fused through a self-cleaving P2A peptide[205] to the N-terminal half of PE2. Nuclei from neurons were isolated two weeks following dual viral transduction and were sequenced directly, or sorted for GFP expression before sequencing. A 7.1±1.2% average prime editing to install a transversion at the DNMT1 locus with 0.58±0.14% average indels in sorted nuclei

(FIG. 43D was observed. Cas9 nuclease in the same split-intein dual lentivirus system resulted in 31±5.5% indels among sorted cortical neuron nuclei (FIG. 43D. These data indicate that post-mitotic, terminally differentiated primary cells can support prime editing, and thus establish that prime editing does not require cell replication.

[1332] Prime editing compared with Cas9-initiated HDR

[1333] The performance of PE3 was compared with that of optimized Cas9-initiated HDR[128,125] in mitotic cell lines that support HDR[128]. HEK293T, HeLa, K562 and U2OS cells were treated with Cas9 nuclease, a sgRNA, and an ssDNA donor oligonucleotide template designed to install a variety of transversion and insertion edits (FIGs. 43E-43G, and FIGs. 51A-51F). Cas9-initiated HDR in all cases successfully installed the desired edit, but with far higher levels of byproducts (predominantly indels), as expected from treatments that cause double-stranded breaks. Using PE3 in HEK293T cells, *HBB* E6V installation and correction proceeded with 42% and 58% average editing efficiency with 2.6% and 1.4% average indels, respectively (FIG. 43E and FIG. 43G). In contrast, the same edits with Cas9 nuclease and an HDR template resulted in 5.2% and 6.7% average editing efficiency, with 79% and 51% average indel frequency (FIG. 43E and FIG. 43G). Similarly, PE3 installed *PRNP* G127V with 53% efficiency and 1.7% indels, whereas Cas9-initiated HDR installed this mutation with 6.9% efficiency and 53% indels (FIG. 43E and FIG. 43G). Thus, the ratio of editing:indels for *HBB* E6V installation, *HBB* E6V correction, and *PRNP* G127V installation on average was 270-fold higher for PE3 than for Cas9-initiated HDR.

[1334] Comparisons between PE3 and HDR in human cell lines other than HEK293T showed similar results, although with lower PE3 editing efficiencies. For example, in K562 cells, PE3-mediated 3-bp insertion into HEK3 proceeded with 25% efficiency and 2.8% indels, compared with 17% editing and 72% indels for Cas9-initiated HDR, a 40-fold editing:indel ratio advantage favoring PE3 (FIGs. 43F-43G). In U2OS cells, PE3 performed this 3-bp insertion with 22% efficiency and 2.2% indels, while Cas9-initiated HDR resulted in 15% editing with 74% indels, a 49-fold lower editing:indel ratio (FIGs. 43F-43G). In HeLa cells, PE3 made this insertion with 12% efficiency and 1.3% indels, versus 3.0% editing and 69% indels for Cas9-initiated HDR, a 210-fold editing:indel ratio difference (FIGs. 43F-43G). Collectively, these data indicated that HDR typically results in similar or lower editing efficiencies and far higher indels than PE3 in the four cell lines tested (FIGs. 51A-51F).

[1335] Discussion and future directions

[1336] The ability to insert DNA sequences with single-nucleotide precision is an especially enabling prime editing capability. For example, PE3 was used to precisely insert into the HEK3 locus in HEK293T cells a His_6 tag (18 bp, 65% average efficiency), a FLAG epitope tag (24 bp, 18% average efficiency), and an extended *LoxP* site (44 bp, 23% average efficiency) that is the native substrate for Cre recombinase. Average indels ranged between 3.0% and 5.9% for these examples (FIG. 43H). Many biotechnological, synthetic biology, and therapeutic applications are envisioned to arise from the ability to efficiently and precisely introduce new DNA sequences into target sites of interest in living cells.

[1337] Collectively, the prime editing experiments described herein installed 18 insertions up to 44 bp, 22 deletions up to 80 bp, 113 point mutations including 77 transversions, and 18 combination edits, across 12 endogenous loci in the human and mouse genomes at locations ranging from 3 bp upstream to 29 bp downstream of the start of a PAM without making explicit double-stranded DNA breaks. These results establish prime editing as a remarkably versatile genome editing method. Because the overwhelming majority (85-99%) of insertions, deletions, indels, and duplications in ClinVar are ≤30 bp (FIGs. 52A-52D), in principle prime editing can correct up to ~89% of the 75,122 currently known pathogenic human genetic variants in ClinVar (transitions, transversions, insertions, deletions, indels, and duplications in FIG. 38A), with additional potential to ameliorate diseases caused by copy number gain or loss.

[1338] Importantly, for any desired edit the flexibility of prime editing offers many possible choices of PEgRNA-induced nick locations, sgRNA-induced second nick locations, PBS lengths, RT template lengths, and which strand to edit first, as demonstrated extensively herein. This flexibility, which contrasts with more limited options typically available for other precision genome editing methods[125,142,154], allows editing efficiency, product purity, DNA specificity, or other parameters to be optimized to suit the needs of a given application, as shown in FIGs. 50A-50B in which testing 14 and 43 PEgRNAs covering a range of prime editing strategies optimized correction of pathogenic HBB and HEXA alleles, respectively.

[1339] Much additional research is needed to further understand and improve prime editing. Additional modifications of prime editor systems may be required to expand their compatibility to include other cell types, such as post-mitotic cells. Interfacing prime editing with viral and non-viral *in vitro* and *in vivo* delivery strategies is needed to fully explore the potential of prime editing to enable a wide range of applications including the study and

treatment of genetic diseases. By enabling highly precise targeted transitions, transversions, small insertions, and small deletions in the genomes of mammalian cells without requiring double- stranded breaks or HDR, however, prime editing provides a new "search-and-replace" capability that substantially expands the scope of genome editing.

Methods

[1340] General methods

[1341] DNA amplification was conducted by PCR using Phusion U Green Multiplex PCR Master Mix (ThermoFisher Scientific) or Q5 Hot Start High-Fidelity 2x Master Mix (New England BioLabs) unless otherwise noted. DNA oligonucleotides, including Cy5- labeled DNA oligonucleotides, dCas9 protein, and Cas9 H840A protein were obtained from Integrated DNA Technologies. Yeast reporter plasmids were derived from previously described plasmids64 and cloned by the Gibson assembly method. All mammalian editor plasmids used herein were assembled using the USER cloning method as previously described[175]. Plasmids expressing sgRNAs were constructed by ligation of annealed oligonucleotides into BsmBI-digested acceptor vector. Plasmids expressing PEgRNAs were constructed by Gibson assembly or Golden Gate assembly using a custom acceptor plasmid (see supplemental 'Golden Gate assembly' outline). Sequences of sgRNA and PEgRNA constructs used herein are listed in Tables 2A-2C and Tables 3A-3R. All vectors for mammalian cell experiments were purified using Plasmid Plus Midiprep kits (Qiagen) or PureYield plasmid miniprep kits (Promega), which include endotoxin removal steps. All experiments using live animals were approved by the Broad Institute Institutional and Animal Care and Use Committees. Wild-type C57BL/6 mice were obtained from Charles River (#027).

[1342] In vitro biochemical assays

[1343] PEgRNAs and sgRNAs were transcribed *in vitro* using the HiScribe T7 *in vitro* transcription kit (New England Biolabs) from PCR-amplified templates containing a T7 promoter sequence. RNA was purified by denaturing urea PAGE and quality- confirmed by an analytical gel prior to use. 5′-Cy5-labeled DNA duplex substrates were annealed using two oligonucleotides (Cy5-AVA024 and AVA025; 1:1.1 ratio) for the non- nicked substrate or three oligonucleotides (Cy5-AVA023, AVA025 and AVA026; 1:1.1:1.1) for the pre-nicked

substrate by heating to 95 °C for 3 minutes followed by slowly cooling to room temperature (Tables 2A-2C). Cas9 cleavage and reverse transcription reactions were carried out in 1x cleavage buffer[205] supplemented with dNTPs (20 mM HEPES-K, pH 7.5; 100 mM KCl; 5% glycerol; 0.2 mM EDTA, pH 8.0; 3 mM MgCl2; 0.5 mM dNTP mix; 5 mM DTT). dCas9 or Cas9 H840A (5 μM final) and the sgRNA or PEgRNA (5 μM final) were pre- incubated at room temperature in a 5 μL reaction mixture for 10 minutes prior to the addition of duplex DNA substrate (400 nM final), followed by the addition of Superscript III reverse transcriptase (ThermoFisher Scientific), an undisclosed M-MLV RT variant, when applicable. Reactions were carried out at 37 °C for 1 hour, then diluted to a volume of 10 μL with water, treated with 0.2 μL of proteinase K solution (20 mg/mL, ThermoFisher Scientific), and incubated at room temperature for 30 minutes. Following heat inactivation at 95 °C for 10 minutes, reaction products were combined with 2x formamide gel loading buffer (90% formamide; 10% glycerol; 0.01% bromophenol blue), denatured at 95 °C for 5 minutes, and separated by denaturing urea-PAGE gel (15% TBE-urea, 55 °C, 200V). DNA products were visualized by Cy5 fluorescence signal using a Typhoon FLA 7000 biomolecular imager.

[1344] Electrophoretic mobility shift assays were carried out in 1x binding buffer (1x cleavage buffer + 10 μg/mL heparin) using pre-incubated dCas9:sgRNA or dCas9:PEgRNA complexes (concentration range between 5 nM and 1 μM final) and Cy5-labeled duplex DNA (Cy5- AVA024 and AVA025; 20 nM final). After 15 minutes of incubation at 37 °C, the samples were analyzed by native PAGE gel (10% TBE) and imaged for Cy5 fluorescence.

[1345] For DNA sequencing of reverse transcription products, fluorescent bands were excised and purified from urea-PAGE gels, then 3′ tailed with terminal transferase (TdT; New England Biolabs) in the presence of dGTP or dATP according to the manufacturer's protocol. Tailed DNA products were diluted 10-fold with binding buffer (40% saturated aqueous guanidinium chloride + 60% isopropanol) and purified by QIAquick spin column (Qiagen), then used as templates for primer extension by Klenow fragment (New England Biolabs) using primer AVA134 (A-tailed products) or AVA135 (G-tailed products) (Tables 2A-2C). Extension were amplified by PCR for 10 cycles using primers AVA110 and AVA122, then sequenced with AVA037 using the Sanger method (Tables 2A-2C).

[1346] Yeast fluorescent reporter assays

[1347] Dual fluorescent reporter plasmids containing an in- frame stop codon, a +1 frameshift, or a -1 frameshift were subjected to 5′-extended PEgRNA or 3′-extended

PEgRNA prime editing reactions *in vitro* as described above. Following incubation at 37 °C for 1 hour, the reactions were diluted with water and plasmid DNA was precipitated with 0.3 M sodium acetate and 70% ethanol. Resuspended DNA was transformed into S. cerevisiae by electroporation as previously described[67] and plated on synthetic complete media without leucine (SC(glucose), L-). GFP and mCherry fluorescence signals were visualized from colonies with the Typhoon FLA 7000 biomolecular imager.

[1348] General mammalian cell culture conditions

[1349] HEK293T (ATCC CRL-3216), U2OS (ATTC HTB-96), K562 (CCL-243), and HeLa (CCL-2) cells were purchased from ATCC and cultured and passaged in Dulbecco's Modified Eagle's Medium (DMEM) plus GlutaMAX (ThermoFisher Scientific), McCoy's 5A Medium (Gibco), RPMI Medium 1640 plus GlutaMAX (Gibco), or Eagle's Minimal Essential Medium (EMEM, ATCC), respectively, each supplemented with 10% (v/v) fetal bovine serum (Gibco, qualified) and 1x Penicillin Streptomycin (Corning). All cell types were incubated, maintained, and cultured at 37 °C with 5% CO_2. Cell lines were authenticated by their respective suppliers and tested negative for mycoplasma.

[1350] HEK293T tissue culture transfection protocol and genomic DNA preparation

[1351] HEK293T cells grown were seeded on 48-well poly-D-lysine coated plates (Corning). 16 to 24 hours post- seeding, cells were transfected at approximately 60% confluency with 1 µL of Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocols and 750 ng of PE plasmid, 250 ng of PEgRNA plasmid, and 83 ng of sgRNA plasmid (for PE3 and PE3b). Unless otherwise stated, cells were cultured 3 days following transfection, after which the media was removed, the cells were washed with 1x PBS solution (Thermo Fisher Scientific), and genomic DNA was extracted by the addition of 150 µL of freshly prepared lysis buffer (10 mM Tris-HCl, pH 7.5; 0.05% SDS; 25 µg/mL Proteinase K (ThermoFisher Scientific)) directly into each well of the tissue culture plate. The genomic DNA mixture was incubated at 37 °C for 1 to 2 hours, followed by an 80 °C enzyme inactivation step for 30 minutes. Primers used for mammalian cell genomic DNA amplification are listed in Table 4. For HDR experiments in HEK293T cells, 231 ng of nuclease-expression plasmid, 69 ng of sgRNA expression plasmid, 50 ng (1.51 pmol) 100-nt ssDNA donor template (PAGE-purified; Integrated DNA Technologies) was lipofected using 1.4 µL Lipofectamine 2000 (ThermoFisher) per well. Genomic DNA from all HDR

experiments was purified using the Agencourt DNAdvance Kit (Beckman Coulter), according to the manufacturer's protocol.

[1352] High-throughput DNA sequencing of genomic DNA samples

[1353] Genomic sites of interest were amplified from genomic DNA samples and sequenced on an Illumina MiSeq as previously described with the following modifications[129,130]. Briefly, amplification primers containing Illumina forward and reverse adapters (Table 4) were used for a first round of PCR (PCR 1) amplifying the genomic region of interest. 25 µL PCR 1 reactions were performed with 0.5 µM of each forward and reverse primer, 1 µL of genomic DNA extract and 12.5 µL of Phusion U Green Multiplex PCR Master Mix. PCR reactions were carried out as follows: 98 °C for 2 minutes, then 30 cycles of [98 °C for 10 seconds, 61 °C for 20 seconds, and 72 °C for 30 seconds], followed by a final 72 °C extension for 2 minutes. Unique Illumina barcoding primer pairs were added to each sample in a secondary PCR reaction (PCR 2). Specifically, 25 µL of a given PCR 2 reaction contained 0.5 µM of each unique forward and reverse illumina barcoding primer pair, 1 µL of unpurified PCR 1 reaction mixture, and 12.5 µL of Phusion U Green Multiplex PCR 2x Master Mix. The barcoding PCR 2 reactions were carried out as follows: 98 °C for 2 minutes, then 12 cycles of [98 °C for 10 seconds, 61 °C for 20 seconds, and 72 °C for 30 seconds], followed by a final 72 °C extension for 2 minutes. PCR products were evaluated analytically by electrophoresis in a 1.5% agarose gel. PCR 2 products (pooled by common amplicons) were purified by electrophoresis with a 1.5% agarose gel using a QIAquick Gel Extraction Kit (Qiagen), eluting with 40 µL of water. DNA concentration was measured by fluorometric quantification (Qubit, ThermoFisher Scientific) or qPCR (KAPA Library Quantification Kit- Illumina, KAPA Biosystems) and sequenced on an Illumina MiSeq instrument according to the manufacturer's protocols.

[1354] Sequencing reads were demultiplexed using MiSeq Reporter (Illumina). Alignment of amplicon sequences to a reference sequence was performed using CRISPResso2[178]. For quantification of point mutation editing, CRISPResso2 was run in standard mode with "discard_indel_reads" on. Editing efficiency was calculated as: (frequency of specified point mutation in non-discarded reads) x (# of non-discarded reads) ÷ total reads. For insertion or deletion edits, CRISPResso2 was run in HDR mode using the desired allele as the expected allele (e flag), and with "discard_indel_reads" ON. Editing yield was calculated as the

number of HDR aligned reads divided by total reads. For all edits, indel yields were calculated as the number of discarded reads divided by total reads.

[1355] Nucleofection of U2OS, K562, and HeLa cells

[1356] Nucleofection was performed in all experiments using K562, HeLa, and U2OS cells. For PE conditions in these cell types, 800ng prime editor-expression plasmid, 200ng PEgRNA-expression plasmid, and 83 ng nicking plasmid was nucleofected in a final volume of 20 µL in a 16-well nucleocuvette strip (Lonza). For HDR conditions in these three cell types, 350 ng nuclease-expression plasmid, 150 ng sgRNA-expression plasmid and 200 pmol (6.6 µg) 100-nt ssDNA donor template (PAGE-purified; Integrated DNA Technologies) was nucleofected in a final volume of 20 µL per sample in a 16-well Nucleocuvette strip (Lonza). K562 cells were nucleofected using the SF Cell Line 4D-Nucleofector X Kit (Lonza) with 5×10^5 cells per sample (program FF-120), according to the manufacturers protocol. U2OS cells were nucleofected using the SE Cell Line 4D-Nucleofector X Kit (Lonza) with 3–4×10^5 cells per sample (program DN-100), according to the manufacturers protocol. HeLa cells were nucleofected using the SE Cell Line 4D-Nucleofector X Kit (Lonza) with 2×10^5 cells per sample (program CN-114), according to the manufacturers protocol. Cells were harvested 72 hours after nucleofection for genomic DNA extraction.

[1357] Genomic DNA extraction for HDR experiments

[1358] Genomic DNA from all HDR comparison experiments in HEK293T, HEK293T *HBB* E6V, K562, U2OS, and HeLa cells was purified using the Agencourt DNAdvance Kit (Beckman Coulter), according to the manufacturer's protocol.

[1359] Comparison between PE2, PE3, BE2, BE4max, ABEdmax, and ABEmax

[1360] HEK293T cells were seeded on 48-well poly-D-lysine coated plates (Corning). After 16 to 24 hours, cells were transfected at approximately 60% confluency. For base editing with CBE or ABE constructs, cells were transfected with 750 ng of base editor plasmid, 250 ng of sgRNA expression plasmid, and 1 µL of Lipofectamine 2000 (Thermo Fisher Scientific). PE transfections were performed as described above. Genomic DNA extraction for PE and BE was performed as described above.

[1361] Determination of PE3 activity at known Cas9 off-target sites

[1362] To evaluate PE3 off-target editing activity at known Cas9 off-target sites, genomic DNA extracted from HEK293T cells 3 days after transfection with PE3 was used as template for PCR amplification of 16 previously reported Cas9 off-target genomic sites[118,159] (the top four off-target sites each for the HEK3, EMX1, FANCF, and HEK4 spacers; primer sequences are listed in Table 4). These genomic DNA samples were identical to those used for quantifying on-target PE3 editing activities shown in FIGs. 41A-41K; PEgRNA and nicking sgRNA sequences are listed in Tables 3A-3R. Following PCR amplification of off-target sites, amplicons were sequenced on the Illumina MiSeq platform as described above (HTS analysis). For determining Cas9 nuclease, Cas9 H840A nickase, dCas9, and PE2-dRT on-target and off- target editing activity, HEK293T cells were transfected with 750 ng of editor plasmid (Cas9 nuclease, Cas9 H840A nickase, dCas9, or PE2-dRT), 250 ng of PEgRNA or sgRNA plasmid, and 1 μL of Lipofectamine 2000. Genomic DNA was isolated from cells 3 days after transfection as described above. On-target and off-target genomic loci were amplified by PCR using primer sequences in Table 4 and sequenced on an Illumina MiSeq.

[1363] HTS data analysis was performed using CRISPResso2[178]. The editing efficiencies of Cas9 nuclease, Cas9 H840A nickase, and dCas9 were quantified as the percent of total sequencing reads containing indels. For quantification of PE3 and PE3-dRT off-targets, aligned sequencing reads were examined for point mutations, insertions, or deletions that were consistent with the anticipated product of PEgRNA reverse transcription initiated at the Cas9 nick site. Single nucleotide variations occurring at <0.1% overall frequency among total reads within a sample were excluded from analysis. For reads containing single nucleotide variations that both occurred at frequencies ≥ 0.1% and were partially consistent with the PEgRNA-encoded edit, t-tests (unpaired, one-tailed, $\alpha = 0.5$) were used to determine if the variants occurred at significantly higher levels compared to samples treated with PEgRNAs that contained the same spacer but encoded different edits. To avoid differences in sequencing errors, comparisons were made between samples that were sequenced simultaneously within the same MiSeq run. Variants that did not meet the criteria of p-value > 0.05 were excluded. Off-target PE3 editing activity was then calculated as the percentage of total sequencing reads that met the above criteria.

[1364] Generation of a HEK293T cell line containing the HBB E6V mutation using Cas9- initiated HDR

[1365] HEK293T cells were seeded in a 48-well plate and transfected at approximately 60% confluency with 1.5 µL of Lipofectamine 2000, 300 ng of Cas9 D10A nickase plasmid, 100 ng of sgRNA plasmid, and 200 ng of 100-mer ssDNA donor template (Table 5). Three days after transfection, media was exchanged for fresh media. Four days after transfection, cells were dissociated using 30 µL of TrypLE solution and suspended in 1.5 mL of media. Single cells were isolated into individual wells of two 96-well plates by fluorescence-activated cell sorting (FACS) (Beckman-Coulter Astrios). See FIGs. 53A-53B for representative FACS sorting examples. Cells were expanded for 14 days prior to genomic DNA sequencing as described above. Of the isolated clonal populations, none was found to be homozygous for the *HBB* E6V mutation, so a second round of editing by lipofection, sorting, and outgrowth was repeated in a partially edited cell line to yield a cell line homozygous for the E6V allele.

[1366] Generation of a HEK293T cell line containing the HBB E6V mutation using PE3

[1367] 2.5 x 104 HEK293T cells grown in the absence of antibiotic were seeded on 48-well poly-D- lysine coated plates (Corning). 16 to 24 hours post-seeding, cells were transfected at approximately 70% confluency with 1 µL of Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's protocols and 750 ng of PE2-P2A-GFP plasmid, 250 ng of PEgRNA plasmid, and 83 ng of sgRNA plasmid. After 3 days post transfection, cells were washed with phosphate-buffered saline (Gibco) and dissociated using TrypLE Express (Gibco). Cells were then diluted with DMEM plus GlutaMax (Thermo Fisher Scientific) supplemented with 10% (v/v) FBS (Gibco) and passed through a 35-µm cell strainer (Corning) prior to sorting. Flow cytometry was carried out on a LE-MA900 cell sorter (Sony). Cells were treated with 3 nM DAPI (BioLegend) 15 minutes prior to sorting. After gating for doublet exclusion, single DAPI- negative cells with GFP fluorescence above that of a GFP-negative control cell population were sorted into 96-well flat-bottom cell culture plates (Corning) filled with pre-chilled DMEM with GlutaMax supplemented with 10% FBS. See FIGs. 53A-53B for representative FACS sorting examples. Cells were cultured for 10 days prior to genomic DNA extraction and characterization by HTS, as described above. A total of six clonal cell lines were identified that are homozygous for the E6V mutation in *HBB*.

[1368] Generation of a HEK293T cell line containing the HEXA 1278+TATC insertion using PE3

[1369] HEK293T cells containing the *HEXA* 1278+TATC allele were generated following the protocol described above for creation of the *HBB* E6V cell line; PEgRNA and sgRNA sequences are listed in Tables 2A-2C under the FIGs. 43A-43H subheading. After transfection and sorting, cells were cultured for 10 days prior to genomic DNA extraction and characterization by HTS, as described above. Two heterozygous cell lines were isolated that contained 50% *HEXA* 1278+TATC alleles, and two homozygous cell lines containing 100% *HEXA* 1278+TATC alleles were recovered.

[1370] Cell viability assays

[1371] HEK293T cells were seeded in 48-well plates and transfected at approximately 70% confluency with 750 ng of editor plasmid (PE3, PE3 R110S K103L, Cas9 H840A nickase, or dCas9), 250 ng of HEK3-targeting PEgRNA plasmid, and 1 μL of Lipofectamine 2000, as described above. Cell viability was measured every 24 hours post-transfection for 3 days using the CellTiter-Glo 2.0 assay (Promega) according to the manufacturer's protocol. Luminescence was measured in 96-well flat-bottomed polystyrene microplates (Corning) using a M1000 Pro microplate reader (Tecan) with a 1-second integration time.

[1372] Lentivirus production

[1373] Lentivirus was produced as previously described[206]. T-75 flasks of rapidly dividing HEK293T cells (ATCC; Manassas, VA, USA) were transfected with lentivirus production helper plasmids pVSV-G and psPAX2 in combination with modified lentiCRISPR_v2 genomes carrying intein-split PE2 editor using FuGENE HD (Promega, Madison, WI, USA) according to the manufacturer's directions. Four split-intein editor constructs were designed: 1) a viral genome encoding a U6-PEgRNA expression cassette and the N-terminal portion (1-573) of Cas9 H840A nickase fused to the Npu N-intein, a self-cleaving P2A peptide, and GFP-KASH; 2) a viral genome encoding the Npu C-intein fused to the C-terminal remainder of PE2; 3) a viral genome encoding the Npu C-intein fused to the C-terminal remainder of Cas9 for the Cas9 control; and 4) a nicking sgRNA for DNMT1. The split-intein mediates trans splicing to join the two halves of PE2 or Cas9, while the P2A GFP-KASH enables co-translational production of a nuclear membrane-localized GFP. After 48 hours, supernatant was collected, centrifuged at 500 g for 5 minutes to remove cellular debris, and filtered using a 0.45 μm filter. Filtered supernatant was concentrated using the PEG-it Virus Precipitation Solution (System Biosciences, Palo Alto, CA, USA) according to the manufacturer's

directions. The resulting pellet was resuspended in Opti-MEM (Thermo Fisher Scientific, Waltham, MA, USA) using 1% of the original media volume. Resuspended pellet was flash-frozen and stored at -80°C until use.

[1374] Mouse primary cortical neuron dissection and culture

[1375] E18.5 dissociated cortical cultures were harvested from timed-pregnant C57BL/6 mice (Charles River). Embryos were harvested from pregnant mice after euthanasia by CO2 followed by decapitation. Cortical caps were dissected in ice-cold Hibernate-E supplemented with penicillin/streptomycin (Life Technologies). Following a rinse with ice-cold Hibernate-E, tissue was digested at 37 °C for 8 minutes in papain/DNase (Worthington/Sigma). Tissue was triturated in NBActiv4 (BrainBits) supplemented with DNase. Cells were counted and plated in 24-well plates at 100,000 cells per well. Half of the media was changed twice per week.

[1376] Prime editing in primary neurons and nuclei isolation

[1377] At DIV 1, 15 µL of lentivirus was added at 10:10:1 ratio of N-terminal:C-terminal:nicking sgRNA. At DIV 14, neuronal nuclei were isolated using the EZ-PREP buffer (Sigma D8938) following the manufacturer's protocol. All steps were performed on ice or at 4 °C. Media was removed from dissociated cultures, and cultures were washed with ice-cold PBS. PBS was aspirated and replaced with 200 µL EZ-PREP solution. Following a 5-minute incubation on ice, EZ-PREP was pipetted across the surface of the well to dislodge remaining cells. The sample was centrifuged at 500 g for 5 minutes, and the supernatant removed. Samples were washed with 200 µL EZ-PREP and centrifuged again at 500 g for 5 minutes. Samples were resuspended with gentle pipetting in 200 µL ice-cold Nuclei Suspension Buffer (NSB) consisting of 100 µg/mL BSA and 3.33 µM Vybrant DyeCycle Ruby (Thermo Fisher) in 1xPBS, then centrifuged at 500 g for 5 minutes. The supernatant was removed and nuclei were resuspended in 100 µL NSB and sorted into 100 µL Agencourt DNAdvance lysis buffer using a MoFlo Astrios (Beckman Coulter) at the Broad Institute flow cytometry facility. Genomic DNA was purified according to the manufacturer's Agencourt DNAdvance instructions.

[1378] RNA-sequencing and data analysis

[1379] HEK293T cells were co-transfected with PRNP-targeting or HEXA-targeting PEgRNAs and PE2, PE2-dRT, or Cas9 H840A nickase. 72 hours following transfection, total RNA was harvested from cells using TRIzol reagent (Thermo Fisher) and purified with RNeasy Mini kit (Qiagen) including on-column DNaseI treatment. Ribosomes were depleted from total RNA using the rRNA removal protocol of the TruSeq Stranded Total RNA library prep kit (Illumina) and subsequently washed with RNAClean XP beads (Beckman Coulter). Sequencing libraries were prepared using ribo-depleted RNA on a SMARTer PrepX Apollo NGS library prep system (Takara) following the manufacturer's protocol. Resulting libraries were visualized on a 2200 TapeStation (Agilent Technologies), normalized using a Qubit dsDNA HS assay (Thermo Fisher), and sequenced on a NextSeq 550 using high output v2 flow cell (Illumina) as 75-bp paired-end reads. Fastq files were generated with bcl2fastq2 version 2.20 and trimmed using TrimGalore version 0.6.2 (https://github.com/FelixKrueger/TrimGalore) to remove low-quality bases, unpaired sequences, and adaptor sequences. Trimmed reads were aligned to a Homo sapiens genome assembly GRCh[148] with a custom Cas9 H840A gene entry using RSEM version 1.3.1[207]. The limma-voom[208] package was used to normalize gene expression levels and perform differential expression analysis with batch effect correction. Differentially expressed genes were called with FDR-corrected p-value < 0.05 and fold-change > 2 cutoffs, and results were visualized in R.

[1380] ClinVar analysis

[1381] The ClinVar variant summary was downloaded from NCBI (accessed July 15, 2019), and the information contained therein was used for all downstream analysis. The list of all reported variants was filtered by allele ID in order to remove duplicates and by clinical significance in order to restrict the analysis to pathogenic variants. The list of pathogenic variants was filtered sequentially by variant type in order to calculate the fraction of pathogenic variants that are insertions, deletions, etc. Single nucleotide variants (SNVs) were separated into two categories (transitions and transversions) based on the reported reference and alternate alleles. SNVs that did not report reference or alternate alleles were excluded from the analysis.

[1382] The lengths of reported insertions, deletions, and duplications were calculated using reference/alternate alleles, variant start/stop positions, or appropriate identifying information in the variant name. Variants that did not report any of the above information were excluded

from the analysis. The lengths of reported indels (single variants that include both insertions and deletions relative to the reference genome) were calculated by determining the number of mismatches or gaps in the best pairwise alignment between the reference and alternate alleles. Frequency distributions of variant lengths were calculated using GraphPad Prism 8.

[1383] Data availability

[1384] High-throughput sequencing data are deposited to the NCBI Sequence Read Archive database. Plasmids encoding PE1, PE2/PE3, and PEgRNA expression vectors will be available from Addgene.

[1385] Code Availability

[1386] The script used to quantify PEgRNA scaffold insertion is provided in FIGs. 60A-60B.

[1387] Supplemental Information: Tables and Sequences

[1388] Table 1: Activities of prime editors, Cas9 nuclease, Cas9 H840A nickase, and PE2-dRT at HEK3, HEK4, EMX1, and FANCF on-target and off-target sites. PE2/PE3 editing is shown as % prime editing alongside % indels (in parentheses). % indels are shown for Cas9, Cas9 H840A nickase (nCas9), and PE2-dRT at the top four previously characterized off-target sites[179,180]. sgRNA and PEgRNA sequences can be found in Tables 3A-3R, under the FIGs. 42A-42H heading. All values are the average of three independent biological replicates.

PE	HEK3 (PE3)					HEK4 (PE2)					EMX1 (PE3)					FANCF (PE3)				
pegRNA / Site	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4
On-target		44.2 (11.9)	51.2 (8.8)	45.4 (16.5)	45.4 (3.3)		18.2 (0.9)	14.4 (1.8)	9.8 (2.0)	7.9 (2.2)		26.6 (3.5)	14.1 (2.4)	35.7 (3.3)	15.4 (2.9)		55.8 (9.3)	32.4 (16.7)	42.8 (13.6)	47.6 (12.0)
Off-target 1		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	0.4 (<0.1)	<0.1 (<0.1)	0.4 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (0.1)	0.6 (0.1)	<0.1 (0.1)	<0.1 (0.1)
Off-target 2		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (0.1)	<0.1 (<0.1)
Off-target 3		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		0.2 (0.5)	0.8 (1.9)	19.2 (0.5)	7.9 (3.5)		<0.1 (0.3)	<0.1 (0.3)	<0.1 (0.3)	<0.1 (0.3)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 4		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (0.1)	<0.1 (0.2)	<0.1 (<0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.2)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)

Cas9	HEK3					HEK4					EMX1					FANCF				
pegRNA / Site	sgRNA	1	2	3	4	sgRNA	1	2	3	4	sgRNA	1	2	3	4	sgRNA	1	2	3	4
On-target	91.3	87.5	89.2	89.1	86.8	71.8	69.6	72.9	72.6	70.9	85.6	79.7	70.6	76.6	76.0	79.7	55.9	58.3	51.8	52.0
Off-target 1	17.2	1.9	5.5	5.2	1.8	54.2	39.5	48.4	49.7	49.2	81.1	63.5	48.1	53.0	59.6	12.6	1.9	1.9	1.7	1.7
Off-target 2	38.0	8.5	12.6	11.8	4.7	42.5	19.5	29.4	27.3	30.3	58.3	12.0	6.0	8.2	12.9	1.1	0.2	0.2	0.2	0.1
Off-target 3	8.8	0.6	1.7	1.5	0.5	98.1	96.9	97.3	97.6	97.5	14.8	4.2	3.1	3.8	4.8	2.4	0.2	<0.1	0.2	0.2
Off-target 4	0.3	<0.1	<0.1	0.1	<0.1	45.3	16.9	28.0	27.5	29.7	39.5	1.3	0.9	0.6	1.3	1.0	0.2	0.2	0.2	0.2

nCas9	HEK3					HEK4					EMX1					FANCF				
pegRNA / Site	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4
Off-target 1		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1		0.1	0.1	<0.1	0.1		<0.1	<0.1	<0.1	<0.1
Off-target 2		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	< 0.1	<0.1		0.3	0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 3		<0.1	<0.1	<0.1	<0.1		0.3	0.5	0.7	0.7		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 4		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	<0.1	<0.1		1.5	0.1	0.1	0.4		<0.1	<0.1	<0.1	<0.1

PE2-dRT	HEK3					HEK4					EMX1					FANCF				
pegRNA / Site	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4	---	1	2	3	4
Off-target 1		<0.1	<0.1	<0.1	<0.1		0.1	0.2	<0.1	<0.1		0.1	0.1	0.1	0.1		<0.1	<0.1	<0.1	<0.1
Off-target 2		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	<0.1	<0.1		<0.1	0.3	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 3		<0.1	<0.1	<0.1	<0.1		<0.1	0.1	1.4	0.9		<0.1	<0.1	<0.1	<0.1		<0.1	<0.1	<0.1	<0.1
Off-target 4		<0.1	<0.1	<0.1	<0.1		0.1	0.1	0.1	0.2		0.9	0.1	0.1	0.2		<0.1	<0.1	<0.1	<0.1

[1389]

[1390] Tables 2A-2C: Sequences of DNA oligonucleotides, PEgRNAs, and sgRNAs used for *in vitro* experiments.

[1391] Table 2A: DNA oligonucleotides

OLIGONUCLEOTIDE	SEQUENCE
AVA023	5CY5-CCTGGGTCAATCCTTGGGGCCCAGACTGAGCACG (SEQ ID NO: 37
AVA024	5CY5-CCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAA (SEQ ID NO: 375)

AVA025	5PHOS-CCTTTCCTCTGCCATCACGTGCTCAGTCTGGGCCCCAAGGATTGACCCA (SEQ ID NO: 376)
AVA026	5PHOS-TGATGGCAGAGGAAAGG (SEQ ID NO: 377)
AVA037	GCAGGCTTTAAAGGAACCAATTC (SEQ ID NO: 378)
AVA110	GCAGGCTTTAAAGGAACCAATTCCCTGGGTCAATCCTTGGGGC (SEQ ID 379)
AVA122	CTCTGGAGGATCTAGCGGAG (SEQ ID NO: 380)
AVA134	CTCTGGAGGATCTAGCGGAGTTTTTTTTTTTTTTTTTT (SEQ ID NO: 38
AVA135	CTCTGGAGGATCTAGCGGAGCCCCCCCCCCCCCC (SEQ ID NO: 382)

[1392] Table 2B: 5′-extended PEgRNAs

PEGRNA	SPACER SEQUENCE	5′ EXTENSION SEQUENCE	LINKER LENGTH (NT)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
PEGRNA 1	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 383)	GGCTAACCGTGCCATTTGATCAGGTCA (SEQ ID NO: 429)	15	5	7
PEGRNA 2	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 384)	GGCTAACCGTGCAAATTAACAAACTAA(SEQ ID NO: 430)	15	5	7
PEGRNA 3	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 385)	GGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 431)	15	5	8
PEGRNA 4	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 386)	GGTCCTCTGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 432)	15	5	15
PEGRNA 5	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 387)	GGCTTCCTTTCCTCTGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 433)	15	5	22
5′-PEGRNA_RT_7_A	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 388)	GGCTAACCGTGCCATTTGATCAGGTCA(SEQ ID NO: 434)	15	5	7
5′-PEGRNA_RT_7_B	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 389)	GGCTAACCGTGCAAATTAACAAACTAA(SEQ ID NO: 435)	15	5	7
5′-PEGRNA_RT_8	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 390)	GGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 436)	15	5	8
5′-PEGRNA_RT_15	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 391)	GGTCCTCTGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 437)	15	5	15

5′-PEGRNA_RT_22	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 392)	GGCTTCCTTTCCTCTGCCATCTCGTGCAAATTAACAAACTAA(SEQ ID NO: 438)	15	5	22

[1393] Table 2C: 3′-extended PEgRNAs

PEGRNA	SPACER SEQUENCE	3′ EXTENSION SEQUENCE	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
3′-PEGRNA_10	GGCCCAGACTGAGCACGTGA(SEQ ID NO: 506)	TCTGCCATCTCGTGCTC (SEQ ID NO: 439)	7	10
3′-PEGRNA_YEAST_TTOA	GGCCCAGACTGAGCACGTGA(SEQ ID NO: 507)	TCTGCCATCTCGTGCTC(SEQ ID NO: 440)	7	10
3′-PEGRNA_YEAST_+1AINS	GGCCCAGACTGAGCACGTGA(SEQ ID NO: 508)	TCTGCCATCATCGTGCTC(SEQ ID NO: 441)	7	11
3′-PEGRNA_YEAST_+1TDEL	GGCCCAGACTGAGCACGTGA(SEQ ID NO: 509)	TCTGCCATCCGTGCTC(SEQ ID NO: 442)	7	9

[1394] Tables 3A-3R: Sequences of PEgRNAs and sgRNAs used in mammalian cell experiments. All sequences are shown in 5′ to 3′ orientation. To construct PEgRNAs, spacer sequences listed below were added to the 5′ end of the sgRNA scaffold and the 3′ extensions listed below containing the primer binding site and RT template were added to the 3′ end of the sgRNA scaffold. The sgRNA scaffold sequence is GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC (SEQ ID NO: 131).

[1395] Table 3A: FIGs. 39A-39D PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NOS: 2890-2996)	3′ EXTENSION (SEQ ID NOS:2997-3103)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_2B-C_8	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCA	8	10
HEK3_2B-C_9	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAG	9	10

HEK3_2B-C_10	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGT	10	10
HEK3_2B-C_11	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTC	11	10
HEK3_2B-C_12	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCT	12	10
HEK3_2B-C_13	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
HEK3_2B-C_14	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGG	14	10
HEK3_2B-C_15	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGG	15	10
HEK3_2C_16	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGGC	16	10
HEK3_2C_17	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTGGGCC	17	10
EMX1_2C_9	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGC	9	13
EMX1_2C_10	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCT	10	13
EMX1_2C_11	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTC	11	13
EMX1_2C_12	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCG	12	13
EMX1_2C_13	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGG	13	13
EMX1_2C_14	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGA	14	13
EMX1_2C_15	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGAC	15	13
EMX1_2C_16	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGACT	16	13
EMX1_2C_17	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGACTC	17	13
FANCF_2C_8	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGA	8	17
FANCF_2C_9	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAA	9	17
FANCF_2C_10	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAG	10	17
FANCF_2C_11	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGG	11	17
FANCF_2C_12	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGG	12	17
FANCF_2C_13	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_2C_14	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGAT	14	17

FANCF_2C_15	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATT	15	17
FANCF_2C_16	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATTC	16	17
FANCF_2C_17	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATTCC	17	17
RNF2_2C_9	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACT	9	11
RNF2_2C_10	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTA	10	11
RNF2_2C_11	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAA	11	11
RNF2_2C_12	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAG	12	11
RNF2_2C_13	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGA	13	11
RNF2_2C_14	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGAT	14	11
RNF2_2C_15	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATG	15	11
RNF2_2C_16	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATGA	16	11
RNF2_2C_17	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATGAC	17	11
HEK4_2C_7	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAG	7	13
HEK4_2C_8	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGC	8	13
HEK4_2C_9	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCC	9	13
HEK4_2C_10	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCG	10	13
HEK4_2C_11	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGC	11	13
HEK4_2C_12	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCA	12	13
HEK4_2C_13	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAG	13	13
HEK4_2C_14	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAGT	14	13
HEK4_2C_15	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGCAGTG	15	13
HEK3_2C_1TDEL	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	10
HEK3_2C_1AINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	10
HEK3_2C_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10

HEK3_2D_10	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
HEK3_2D_11	GGCCCAGACTGAGCACGTGA	CTCTGCCATCTCGTGCTCAGTCTG	13	11
HEK3_2D_12	GGCCCAGACTGAGCACGTGA	CCTCTGCCATCTCGTGCTCAGTCTG	13	12
HEK3_2D_13	GGCCCAGACTGAGCACGTGA	TCCTCTGCCATCTCGTGCTCAGTCTG	13	13
HEK3_2D_14	GGCCCAGACTGAGCACGTGA	TTCCTCTGCCATCTCGTGCTCAGTCTG	13	14
HEK3_2D_15	GGCCCAGACTGAGCACGTGA	TTTCCTCTGCCATCTCGTGCTCAGTCTG	13	15
HEK3_2D_16	GGCCCAGACTGAGCACGTGA	CTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	16
HEK3_2D_17	GGCCCAGACTGAGCACGTGA	CCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	17
HEK3_2D_18	GGCCCAGACTGAGCACGTGA	TCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	18
HEK3_2D_19	GGCCCAGACTGAGCACGTGA	TTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	19
HEK3_2D_20	GGCCCAGACTGAGCACGTGA	CTTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	20
EMX1_2D_10	GAGTCCGAGCAGAAGAAGAA	GGAGCCCTTGTTCTTCTGCTCGG	13	10
EMX1_2D_11	GAGTCCGAGCAGAAGAAGAA	GGGAGCCCTTGTTCTTCTGCTCGG	13	11
EMX1_2D_12	GAGTCCGAGCAGAAGAAGAA	TGGGAGCCCTTGTTCTTCTGCTCGG	13	12
EMX1_2D_13	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCCCTTGTTCTTCTGCTCGG	13	13
EMX1_2D_14	GAGTCCGAGCAGAAGAAGAA	GATGGGAGCCCTTGTTCTTCTGCTCGG	13	14
EMX1_2D_15	GAGTCCGAGCAGAAGAAGAA	TGATGGGAGCCCTTGTTCTTCTGCTCGG	13	15
EMX1_2D_16	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	16
EMX1_2D_17	GAGTCCGAGCAGAAGAAGAA	TGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	17
EMX1_2D_18	GAGTCCGAGCAGAAGAAGAA	ATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	18
EMX1_2D_19	GAGTCCGAGCAGAAGAAGAA	GATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	19
EMX1_2D_20	GAGTCCGAGCAGAAGAAGAA	TGATGTGATGGGAGCCCTTGTTCTTCTGCTCGG	13	20
FANCF_2D_10	GGAATCCCTTCTGCAGCACC	CGATCAAGGTGCTGCAGAAGGGA	13	10
FANCF_2D_11	GGAATCCCTTCTGCAGCACC	GCGATCAAGGTGCTGCAGAAGGGA	13	11

FANCF_2D_12	GGAATCCCTTCTGCAGCACC	AGCGATCAAGGTGCTGCAGAAGGGA	13	12
FANCF_2D_13	GGAATCCCTTCTGCAGCACC	AAGCGATCAAGGTGCTGCAGAAGGGA	13	13
FANCF_2D_14	GGAATCCCTTCTGCAGCACC	AAAGCGATCAAGGTGCTGCAGAAGGGA	13	14
FANCF_2D_15	GGAATCCCTTCTGCAGCACC	AAAAGCGATCAAGGTGCTGCAGAAGGGA	13	15
FANCF_2D_16	GGAATCCCTTCTGCAGCACC	GAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	16
FANCF_2D_17	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_2D_18	GGAATCCCTTCTGCAGCACC	CGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	18
FANCF_2D_19	GGAATCCCTTCTGCAGCACC	TCGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	19
FANCF_2D_20	GGAATCCCTTCTGCAGCACC	CTCGGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	20
RNF2_2D_10	GTCATCTTAGTCATTACCTG	AACACCTCATGTAATGACTAAGATG	15	10
RNF2_2D_11	GTCATCTTAGTCATTACCTG	GAACACCTCATGTAATGACTAAGATG	15	11
RNF2_2D_12	GTCATCTTAGTCATTACCTG	CGAACACCTCATGTAATGACTAAGATG	15	12
RNF2_2D_13	GTCATCTTAGTCATTACCTG	ACGAACACCTCATGTAATGACTAAGATG	15	13
RNF2_2D_14	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_2D_15	GTCATCTTAGTCATTACCTG	CAACGAACACCTCATGTAATGACTAAGATG	15	15
RNF2_2D_16	GTCATCTTAGTCATTACCTG	ACAACGAACACCTCATGTAATGACTAAGATG	15	16
RNF2_2D_17	GTCATCTTAGTCATTACCTG	TACAACGAACACCTCATGTAATGACTAAGATG	15	17
RNF2_2D_18	GTCATCTTAGTCATTACCTG	TTACAACGAACACCTCATGTAATGACTAAGATG	15	18
RNF2_2D_19	GTCATCTTAGTCATTACCTG	GTTACAACGAACACCTCATGTAATGACTAAGATG	15	19
RNF2_2D_20	GTCATCTTAGTCATTACCTG	AGTTACAACGAACACCTCATGTAATGACTAAGATG	15	20
HEK4_2D_7	GGCACTGCGGCTGGAGGTGG	ACCCCAACCTCCAGCCGC	11	7
HEK4_2D_8	GGCACTGCGGCTGGAGGTGG	AACCCCAACCTCCAGCCGC	11	8
HEK4_2D_9	GGCACTGCGGCTGGAGGTGG	TAACCCCAACCTCCAGCCGC	11	9
HEK4_2D_10	GGCACTGCGGCTGGAGGTGG	TTAACCCCAACCTCCAGCCGC	11	10

HEK4_2D_11	GGCACTGCGGCTGGAGGTGG	TTTAACCCCAACCTCCAGCCGC	11	11
HEK4_2D_12	GGCACTGCGGCTGGAGGTGG	CTTTAACCCCAACCTCCAGCCGC	11	12
HEK4_2D_13	GGCACTGCGGCTGGAGGTGG	GCTTTAACCCCAACCTCCAGCCGC	11	13
HEK4_2D_14	GGCACTGCGGCTGGAGGTGG	CGCTTTAACCCCAACCTCCAGCCGC	11	14
HEK4_2D_15	GGCACTGCGGCTGGAGGTGG	CCGCTTTAACCCCAACCTCCAGCCGC	11	15
HEK4_2D_16	GGCACTGCGGCTGGAGGTGG	TCCGCTTTAACCCCAACCTCCAGCCGC	11	16
HEK4_2D_17	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	17
HEK4_2D_18	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	18
HEK4_2D_19	GGCACTGCGGCTGGAGGTGG	CTCCGCTTTAACCCCAACCTCCAGCCGC	11	19

[1396] Table 3B: FIGs. 40A-40C PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 3104-3112)	3′ EXTENSION (SEQ ID NOS: 3113-3121)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
RNF2_3B	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
EMX1_3B	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCACTTCTTCTTCTGCTCGGAC	15	13
FANCF_3B	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGATT	15	17
HE3_3B	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK4_3B	GGCACTGCGGCTGGAGGTGG	TTAACCCCAACCTCCAGCC	9	10
RNF2_3C_4ATOC	GTCATCTTAGTCATTACCTG	AACGAACACCGCAGGTAATGACTAAGATG	15	14
RNF2_3C_4ATOG	GTCATCTTAGTCATTACCTG	AACGAACACCCCAGGTAATGACTAAGATG	15	14
FANCF_3C_5GTOT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGGTGCTGCAGAAGGGA	13	17
FANCF_3C_7ATOC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAGCCAGGTGCTGCAGAAGGGAT	14	17

[1397] Table 3C: FIGs. 40A-40C nicking sgRNA sequences

NICKING SGRNA	SPACER SEQUENCE	SEQ ID NO:
RNF2_2B_+41	GTCAACCATTAAGCAAAACAT	**3122**
RNF2_2B_+67	GTCTCAGGCTGTGCAGACAAA	**3123**
EMX1_2B_-116	GGGGCACAGATGAGAAACTC	**3124**
EMX1_2B_-57	GCCGTTTGTACTTTGTCCTC	**3125**
EMX1_2B_+14	GCGCCACCGGTTGATGTGAT	**3126**
EMX1_2B_+27	GCTTCGTGGCAATGCGCCAC	**3127**
EMX1_2B_+53	GACATCGATGTCCTCCCCAT	**3128**
EMX1_2B_+80	GTGGTTGCCCACCCTAGTCAT	**3129**
FANCF_2B_-78	GCGACTCTCTGCGTACTGAT	**3130**
FANCF_2B_-50	GCCCTACTTCCGCTTTCACCT	**3131**
FANCF_2B_-27	GGATTCCATGAGGTGCGCGA	**3132**
FANCF_2B_-17	GCTGCAGAAGGGATTCCATG	**3133**
FANCF_2B_+21	GCTTGAGACCGCCAGAAGCT	**3134**
FANCF_2B_+48	GGGGTCCCAGGTGCTGACGT	**3135**
HEK3_2B_-108	GCAGAAATAGACTAATTGCA	**3136**
HEK3_2B_-38	GGATTGACCCAGGCCAGGGC	**3137**
HEK3_2B_+26	GACGCCCTCTGGAGGAAGCA	**3138**
HEK3_2B_+37	GCTGTCCTGCGACGCCCTC	**3139**
HEK3_2B_+63	GCACATACTAGCCCCTGTCT	**3140**
HEK3_2B_+90	GTCAACCAGTATCCCGGTGC	**3141**
HEK4_2B_-95	TCCCTTCCTTCCACCCAGCC	**3142**
HEK4_2B_-51	CCCTGCCTGTCATCCTGCTT	**3143**
HEK4_2B_-26	GCAGTGCCACCGGGGCGCCG	**3144**
HEK4_2B_+52	GCGGGGGCTCAGAGAGGGCA	**3145**
HEK4_2B_+74	GAGACACACACACAGGCCTGG	**3146**
RNF2_2C_+41	GTCAACCATTAAGCAAAACAT	**3147**
RNF2_2C_4ATOC_+5	GTGAGTTACAACGAACACCGC	**3148**
RNF2_2C_4ATOG_+5	GTGAGTTACAACGAACACCCC	**3149**
FANCF_2C_+48	GGGGTCCCAGGTGCTGACGT	**3150**
FANCF_2C_5GTOT_+7	GAAGCTCGGAAAAGCGATCA	**3151**
FANCF_2C_7ATOC_+7	GAAGCTCGGAAAAGCGAGCC	**3152**
HEK3_2C_+90	GTCAACCAGTATCCCGGTGC	**3153**

[1398] Table 3D: FIGs. 41A-41K PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 3154-3304)	3′ EXTENSION (SEQ ID NO: 3305-3455)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_4A_1TTOA	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
HEK3_4A_1TTOC	GGCCCAGACTGAGCACGTGA	TCTGCCATCGCGTGCTCAGTCTG	13	10
HEK3_4A_1TTOG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10

HEK3_4A_2GTOA	GGCCCAGACTGAGCACGTGA	TCTGCCATTACGTGCTCAGTCTG	13	10
HEK3_4A_2GTOC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK3_4A_2GTOT	GGCCCAGACTGAGCACGTGA	TCTGCCATAACGTGCTCAGTCTG	13	10
HEK3_4A_3ATOC	GGCCCAGACTGAGCACGTGA	TCTGCCAGCACGTGCTCAGTCTG	13	10
HEK3_4A_3ATOG	GGCCCAGACTGAGCACGTGA	TCTGCCACCACGTGCTCAGTCTG	13	10
HEK3_4A_3ATOT	GGCCCAGACTGAGCACGTGA	TCTGCCAACACGTGCTCAGTCTG	13	10
HEK3_4A_4TTOA	GGCCCAGACTGAGCACGTGA	TCTGCCTTCACGTGCTCAGTCTG	13	10
HEK3_4A_4TTOC	GGCCCAGACTGAGCACGTGA	TCTGCCGTCACGTGCTCAGTCTG	13	10
HEK3_4A_4TTOG	GGCCCAGACTGAGCACGTGA	TCTGCCCTCACGTGCTCAGTCTG	13	10
HEK3_4A_5GTOA	GGCCCAGACTGAGCACGTGA	TCTGCTATCACGTGCTCAGTCTG	13	10
HEK3_4A_5GTOC	GGCCCAGACTGAGCACGTGA	TCTGCGATCACGTGCTCAGTCTG	13	10
HEK3_4A_5GTOT	GGCCCAGACTGAGCACGTGA	TCTGCAATCACGTGCTCAGTCTG	13	10
HEK3_4A_6GTOA	GGCCCAGACTGAGCACGTGA	TCTGTCATCACGTGCTCAGTCTG	13	10
HEK3_4A_6GTOC	GGCCCAGACTGAGCACGTGA	TCTGGCATCACGTGCTCAGTCTG	13	10
HEK3_4A_6GTOT	GGCCCAGACTGAGCACGTGA	TCTGACATCACGTGCTCAGTCTG	13	10
HEK3_4A_7CTOA	GGCCCAGACTGAGCACGTGA	TCTTCCATCACGTGCTCAGTCTG	13	10
HEK3_4A_7CTOG	GGCCCAGACTGAGCACGTGA	TCTCCCATCACGTGCTCAGTCTG	13	10
HEK3_4A_7CTOT	GGCCCAGACTGAGCACGTGA	TCTACCATCACGTGCTCAGTCTG	13	10
HEK3_4A_8ATOC	GGCCCAGACTGAGCACGTGA	TCGGCCATCACGTGCTCAGTCTG	13	10
HEK3_4A_8ATOG	GGCCCAGACTGAGCACGTGA	TCCGCCATCACGTGCTCAGTCTG	13	10
HEK3_4A_8ATOT	GGCCCAGACTGAGCACGTGA	TCAGCCATCACGTGCTCAGTCTG	13	10
HEK3_4B_1TTOA	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCTCGTGCTCAGTCTG	13	34
HEK3_4B_12GTOC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTGCTCTGCCATCACGTGCTCAGTCTG	13	34

HEK3_4B_14ATOT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTATCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_17GTOC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTGCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_20GTOC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGGTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_23CTOG	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCACGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_24TTOA	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCTGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_26CTOG	GGCCCAGACTGAGCACGTGA	TGGAGGAACCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_30CTOG	GGCCCAGACTGAGCACGTGA	TGGACGAAGCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
HEK3_4B_33CTOG	GGCCCAGACTGAGCACGTGA	TCGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACGTGCTCAGTCTG	13	34
RNF2_4C_1CTOA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_4C_1CTOG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
RNF2_4C_1CTOT	GTCATCTTAGTCATTACCTG	AACGAACACCTCAAGTAATGACTAAGATG	15	14
RNF2_4C_2TTOA	GTCATCTTAGTCATTACCTG	AACGAACACCTCTGGTAATGACTAAGATG	15	14
RNF2_4C_2TTOG	GTCATCTTAGTCATTACCTG	AACGAACACCTCCGGTAATGACTAAGATG	15	14
RNF2_4C_3GTOC	GTCATCTTAGTCATTACCTG	AACGAACACCTGAGGTAATGACTAAGATG	15	14
RNF2_4C_4ATOC	GTCATCTTAGTCATTACCTG	AACGAACACCGCAGGTAATGACTAAGATG	15	14
RNF2_4C_4ATOT	GTCATCTTAGTCATTACCTG	AACGAACACCACAGGTAATGACTAAGATG	15	14
RNF2_4C_4ATOG	GTCATCTTAGTCATTACCTG	AACGAACACCCCAGGTAATGACTAAGATG	15	14
RNF2_4C_5GTOT	GTCATCTTAGTCATTACCTG	AACGAACACATCAGGTAATGACTAAGATG	15	14
RNF2_4C_6GTOA	GTCATCTTAGTCATTACCTG	AACGAACATCTCAGGTAATGACTAAGATG	15	14
RNF2_4C_7TTOC	GTCATCTTAGTCATTACCTG	AACGAACGCCTCAGGTAATGACTAAGATG	15	14

FANCF_4D_1ATOG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGCGCTGCAGAAGGGAT	14	17
FANCF_4D_1ATOT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGAGCTGCAGAAGGGAT	14	17
FANCF_4D_2CTOA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGTTGCTGCAGAAGGGAT	14	17
FANCF_4D_3CTOG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCACGTGCTGCAGAAGGGAT	14	17
FANCF_4D_3CTOT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAAGTGCTGCAGAAGGGAT	14	17
FANCF_4D_4TTOA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCTGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_4TTOG	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCCGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_5GTOA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCTAGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_6GTOC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATGCAGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_7ATOC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAGCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_8TTOC	GGAATCCCTTCTGCAGCACC	GGAAAAGCGGTCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_4D_10GTOT	GGAATCCCTTCTGCAGCACC	GGAAAAGAGATCCAGGTGCTGCAGAAGGGAT	14	17
EMX1_4E_2ATOC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTGCTTCTTCTGCTCGGA	14	16
EMX1_4E_2ATOT	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTACTTCTTCTGCTCGGA	14	16
EMX1_4E_3ATOG	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCCTCTTCTTCTGCTCGGA	14	16
EMX1_4E_4GTOC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCGTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_5GTOA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_5GTOT	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_7CTOA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGATCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_8TTOA	GAGTCCGAGCAGAAGAAGAA	GTGATGGGTGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_8TTOC	GAGTCCGAGCAGAAGAAGAA	GTGATGGGGGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_8TTOG	GAGTCCGAGCAGAAGAAGAA	GTGATGGGCGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_9CTOG	GAGTCCGAGCAGAAGAAGAA	GTGATGGCAGCCCTTCTTCTTCTGCTCGGA	14	16
EMX1_4E_9CTOT	GAGTCCGAGCAGAAGAAGAA	GTGATGGAAGCCCTTCTTCTTCTGCTCGGA	14	16

RUNX1_4F_1CTOA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCTCTTCCTCCTGAAAAT	15	15
RUNX1_4F_1CTOG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCCCTTCCTCCTGAAAAT	15	15
RUNX1_4F_1CTOT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCACTTCCTCCTGAAAAT	15	15
RUNX1_4F_2GTOA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATTGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_3ATOC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCAGCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_3ATOG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCACCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_3ATOT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCAACGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_4TTOA	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCTTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_4TTOC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCGTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_4TTOG	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCCTCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_5GTOT	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	15
RUNX1_4F_6GTOC	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGGCATCGCTTCCTCCTGAAAAT	15	15
VEGFA_4G_1TTOA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCTTCTGGCCTGCAGA	13	22
VEGFA_4G_1TTOC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCGTCTGGCCTGCAGA	13	22
VEGFA_4G_1TTOG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTCCTCTGGCCTGCAGA	13	22
VEGFA_4G_2GTOA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTTATCTGGCCTGCAGA	13	22
VEGFA_4G_3ATOC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCGCATCTGGCCTGCAGA	13	22
VEGFA_4G_3ATOG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCCCATCTGGCCTGCAGA	13	22
VEGFA_4G_3ATOT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCACATCTGGCCTGCAGA	13	22
VEGFA_4G_5GTOT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	22
VEGFA_4G_6GTOC	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGGCCTCATCTGGCCTGCAGA	13	22
VEGFA_4G_7CTOA	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGATCCCTCATCTGGCCTGCAGA	13	22
VEGFA_4G_7CTOT	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAACCCTCATCTGGCCTGCAGA	13	22
VEGFA_4G_9CTOG	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGCAGCCCTCATCTGGCCTGCAGA	13	22

DNMT1_4H_1ATOC	GATTCCTGGTGCCAGAAACA	GTCACCCCTGGTTCTGGCACCAGG	13	11
DNMT1_4H_1ATOG	GATTCCTGGTGCCAGAAACA	GTCACCCCTGCTTCTGGCACCAGG	13	11
DNMT1_4H_2CTOA	GATTCCTGGTGCCAGAAACA	GTCACCCCTTTTTCTGGCACCAGG	13	11
DNMT1_4H_2CTOG	GATTCCTGGTGCCAGAAACA	GTCACCCCTCTTTCTGGCACCAGG	13	11
DNMT1_4H_2CTOT	GATTCCTGGTGCCAGAAACA	GTCACCCCTATTTCTGGCACCAGG	13	11
DNMT1_4H_3ATOT	GATTCCTGGTGCCAGAAACA	GTCACCCCAGTTTCTGGCACCAGG	13	11
DNMT1_4H_4GTOA	GATTCCTGGTGCCAGAAACA	GTCACCCTTGTTTCTGGCACCAGG	13	11
DNMT1_4H_5GTOT	GATTCCTGGTGCCAGAAACA	GTCACCACTGTTTCTGGCACCAGG	13	11
DNMT1_4H_6GTOC	GATTCCTGGTGCCAGAAACA	GTCACGCCTGTTTCTGGCACCAGG	13	11
DNMT1_4H_8TTOA	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCTCCCCTGTTTCTGGCACCAGG	13	19
DNMT1_4H_8TTOC	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCGCCCCTGTTTCTGGCACCAGG	13	19
DNMT1_4H_8TTOG	GATTCCTGGTGCCAGAAACA	GCCCTCCCGTCCCCCCTGTTTCTGGCACCAGG	13	19
HEK3_4J_DEL1-5	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCGTGCTCAGTCTG	13	29
HEK3_4J_DEL1-10	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCCGTGCTCAGTCTG	13	24
HEK3_4J_DEL1-15	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCCGTGCTCAGTCTG	13	19
HEK3_4J_DEL1-25	GGCCCAGACTGAGCACGTGA	TGTCCTGCGACGCCCTCTGGAGGAAGCGTGCTCAGTCTG	13	26
HEK3_4J_DEL1-30	GGCCCAGACTGAGCACGTGA	TGTCCTGCGACGCCCTCTGGACGTGCTCAGTCTG	13	21
HEK3_4J_DEL1-80	GGCCCAGACTGAGCACGTGA	AGTATCCCGGTGCAGGAGCTCGTGCTCAGTCTG	13	20
HEK3_4I_1AINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	11
HEK3_4I_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
HEK3_4I_1TDEL	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
HEK3_4I_1-3TGADEL	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCACGTGCTCAGTCTG	13	31

RNF2_4I_1TINS	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGAGTAATGACTAAGATG	15	15
RNF2_4I_1GTAINS	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGTACGTAATGACTAAGATG	15	17
RNF2_4I_4ADEL	GTCATCTTAGTCATTACCTG	AACGAACACCCAGGTAATGACTAAGATG	15	13
RNF2_4I_3-5GAGDEL	GTCATCTTAGTCATTACCTG	AACGAACACAGGTAATGACTAAGATG	15	11
FANCF_4I_3CINS	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGGTGCTGCAGAAGGGAT	14	18
FANCF_4I_4GATINS	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAATCGGTGCTGCAGAAGGGAT	14	20
FANCF_4I_6GDEL	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAGGTGCTGCAGAAGGGAT	14	16
FANCF_4I_5-7GGADEL	GGAATCCCTTCTGCAGCACC	GGAAAAGCGAAGGTGCTGCAGAAGGGAT	14	14
EMX1_4I_6TINS	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACCTTCTTCTTCTGCTCGGA	14	17
EMX1_4I_1TGCINS	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTCGCATTCTTCTGCTCGGA	14	19
EMX1_4I_5GDEL	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCTTCTTCTTCTGCTCGGA	14	15
EMX1_4I_4-6GGGDEL	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTGCTCGGA	14	13
RUNX1_4I_1CINS	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCGGCTTCCTCCTGAAAAT	15	16
RUNX1_4I_1ATGINS	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATCCATGCTTCCTCCTGAAAAT	15	18
RUNX1_4I_2GDEL	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCATGCTTCCTCCTGAAAAT	15	14
RUNX1_4I_2-4GATDEL	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCCGCTTCCTCCTGAAAAT	15	12
VEGFA_4I_4CINS	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCGCTCATCTGGCCTGCAGA	13	23
VEGFA_4I_2ACAINS	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCTTGTCATCTGGCCTGCAGA	13	25
VEGFA_4I_3ADEL	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCCCATCTGGCCTGCAGA	13	21
VEGFA_4I_2-4GAGDEL	GATGTCTGCAGGCCAGATGA	AATGTGCCATCTGGAGCCATCTGGCCTGCAGA	13	19
DNMT1_4I_4CINS	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCGCTGTTTCTGGCACCAGG	13	16
DNMT1_4I_1TCAINS	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCCTGTGATTTCTGGCACCAGG	13	18

DNMT1_4I_3ADEL	GATTCCTGGTGCCAGAAACA	TCCCGTCACCCCGTTTCTGGCACCAGG	13	14
DNMT1_4I_3-5AGGDEL	GATTCCTGGTGCCAGAAACA	TCCCGTCACCGTTTCTGGCACCAGG	13	12
HEK3_4K_1CTTINS_5GDEL	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCATCAAAGCGTGCTCAGTCTG	13	36
HEK3_4K_1CTTINS_2GTOC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATGAAAGCGTGCTCAGTCTG	13	37
HEK3_4K_3TDEL_5GTOC	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCGATCCGTGCTCAGTCTG	13	33
HEK3_4K_3GTOC_6GTOT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGACATGACGTGCTCAGTCTG	13	34
RNF2_4K_2AAINS_3-4GADEL	GTCATCTTAGTCATTACCTG	AACGAACACCATTGGTAATGACTAAGATG	15	14
RNF2_4K_1AINS_5GTOC	GTCATCTTAGTCATTACCTG	AACGAACACGTCAGTGTAATGACTAAGATG	15	15
RNF2_4K_1-2CTDEL_6GTOT	GTCATCTTAGTCATTACCTG	AACGAACAACTCGTAATGACTAAGATG	15	12
RNF2_4K_1CTOA_5GTOT	GTCATCTTAGTCATTACCTG	AACGAACACATCATGTAATGACTAAGATG	15	14
FANCF_4K_1TINS_3-4TGDEL	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCGGTAGCTGCAGAAGGGAT	14	16
FANCF_4K_1TINS_6GTOA	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATTCAGGTAGCTGCAGAAGGGAT	14	18
FANCF_4K_2CDEL_5GTOT	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCAAGTGCTGCAGAAGGGAT	14	16

[1399]

[1400] Table 3E: FIGs. 41A-41K nicking sgRNA

NICKING SGRNA	SPACER SEQUENCE (SEQ ID NOS: 3456-3463)
HEK3_4A_+90	GTCAACCAGTATCCCGGTGC
HEK3_4B_+90	GTCAACCAGTATCCCGGTGC
RNF2_4C_+41	GTCAACCATTAAGCAAAACAT
FANCF_4D_+48	GGGGTCCCAGGTGCTGACGT

EMX1_4E_+53	GACATCGATGTCCTCCCCAT
RUNX1_4F_+38	GATGAAGCACTGTGGGTACGA
VEGFA_4G_+57	GATGTACAGAGAGCCCAGGGC
DNMT1_4H_+49	GCCCTTCAGCTAAAATAAAGG

[1401]

[1402] Table 3F: FIGs. 42A-42H PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NOS: 3464-3478)	3′ EXTENSION (SEQ ID NOS: 3479-3493)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_5A_C3	GGCCCAGACTGAGCACGTGA	TCTGTCATCACGTGCTCAGTCTG	13	10
HEK3_5A_C4	GGCCCAGACTGAGCACGTGA	TCTGCTATCACGTGCTCAGTCTG	13	10
HEK3_5A_C7	GGCCCAGACTGAGCACGTGA	TCTGCCATTACGTGCTCAGTCTG	13	10
FANCF_5A_C3	GGAATCCCTTCTGCAGCACC	GGAAAAGTGATCCAGGTGCTGCAGAAGGGAT	14	17
FANCF_5A_C7	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATTCAGGTGCTGCAGAAGGGAT	14	17
FANCF_5A_C8	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCTAGGTGCTGCAGAAGGGAT	14	17
EMX1_5A_C5	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTCCTTCTTCTTCTGCTCGGA	14	16
EMX1_5A_C6	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTCTTCTTCTTCTGCTCGGA	14	16
EMX1_5A_C7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCTTTCTTCTTCTGCTCGGA	14	16
EMX1_5C_C5_6	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTTCTGCTCGGA	14	16
EMX1_5C_C5_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTCTTTCTTCTTCTGCTCGGA	14	16
EMX1_5C_C6_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCTTTTCTTCTTCTGCTCGGA	14	16
EMX1_5C_C5_6_7	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTTTTCTTCTTCTGCTCGGA	14	16
HEK3_5D_A5	GGCCCAGACTGAGCACGTGA	TCTGCCGTCACGTGCTCAGTCTG	13	10
HEK3_5D_A8	GGCCCAGACTGAGCACGTGA	TCTGCCATCGCGTGCTCAGTCTG	13	10

[1403] Table 3G: FIGs. 42A-42H nicking sgRNA

NICKING SGRNA	POSSIBLE SPACER SEQUENCE (SEQ ID NOS: 616-618)	POSSIBLE SPACER SEQUENCE(SEQ ID NOS: 619-621)

HEK3_5A-F_+90	GTCAACCAGTATCCCGGTGC	GTCAACCAGTATCCCGGTGC
FANCF_5A-F_+48	GGGGTCCCAGGTGCTGACGT	GATGTACAGAGAGCCCAGGGC
EMX1_5A-F_+57	GATGTACAGAGAGCCCAGGGC	GGGGTCCCAGGTGCTGACGT

[1404] Table 3H: FIGs. 42A-42H base editing sgRNA

BASE EDITING SGRNA	SPACER SEQUENCE
HEK3_5A-F_BE	GTGCCATCACGTGCTCAGTCT (SEQ ID NO: 455)
FANCF_5A-F_BE	GAGCGATCCAGGTGCTGCAGA (SEQ ID NO: 456)
EMX1_5A-F_BE	GGAGCCCTTCTTCTTCTGCT (SEQ ID NO: 455)

[1405] Table 3I: FIGs. 42A-42H on-target sgRNA

ON-TARGET SGRNA	SPACER SEQUENCE
HEK3_5G	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 510)
HEK4_5G	GGCACTGCGGCTGGAGGTGG (SEQ ID NO: 511)
EMX1_5G	GAGTCCGAGCAGAAGAAGAA (SEQ ID NO: 512)
FANCF_5G	GGAATCCCTTCTGCAGCACC (SEQ ID NO: 513)

[1406] Table 3J: FIGs. 42A-42H on-target PEgRNA

ON-TARGET PEGRNA	SPACER SEQUENCE (SEQ ID NO: 663-677)	3′ EXTENSION (SEQ ID NO: 678-692)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_5G-H_PEGRNA_1	GGCCCAGACTGAGCACGTGA	TCTGCCATCTCGTGCTCAGTCTG	13	10
HEK3_5G-H_PEGRNA_2	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
HEK3_5G-H_PEGRNA_3	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
HEK3_5G-H_PEGRNA_4	GGCCCAGACTGAGCACGTGA	TCTGCGATCACGTGCTCAGTCTG	13	10
HEK4_5G-H_PEGRNA_1	GGCACTGCGGCTGGAGGTGG	TTAACGCCCACCTCCAGCC	9	10
HEK4_5G-H_PEGRNA_2	GGCACTGCGGCTGGAGGTGG	TTAACCCCCCCCTCCAGCC	9	10

HEK4_5G-H_PEGRNA_3	GGCACTGCGGCTGGAGGTGG	TTAACCCCTTACACCTCCAGCC	9	13
HEK4_5G-H_PEGRNA_4	GGCACTGCGGCTGGAGGTGG	TTAACCCCCCCTCCAGCC	9	9
EMX1_5G-H_PEGRNA_1	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCACTTCTTCTTCTGCTCGGA	14	16
EMX1_5G-H_PEGRNA_2	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTGCTTCTTCTGCTCGGA	14	16
EMX1_5G-H_PEGRNA_3	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGCCCTTCGCATTCTTCTGCTCGGA	14	19
EMX1_5G-H_PEGRNA_4	GAGTCCGAGCAGAAGAAGAA	GTGATGGGAGTTCTTCTTCTGCTCGGA	14	13
FANCF_5G-H_PEGRNA_1	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATGCAGGTGCTGCAGAAGGGAT	14	17
FANCF_5G-H_PEGRNA_2	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAGGCGCTGCAGAAGGGAT	14	17
FANCF_5G-H_PEGRNA_3	GGAATCCCTTCTGCAGCACC	GGAAAAGCGATCCAATCGGTGCTGCAGAAGGGAT	14	20

[1407] Table 3K: FIGs. 49A-49BPEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 3494-3521)	3′ EXTENSION (SEQ ID NO: 3522-3540)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_6A_2GTOC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK3_6A_2GTOC	GGCCCAGACTGAGCACGTGA			
EMX1_6A_3GTOC	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCCCTTGTTCTTCTGCTCGG	13	13
EMX1_6A_3GTOC	GAGTCCGAGCAGAAGAAGAA			
FANCF_6A_5GTOT	GGAATCCCTTCTGCAGCACC	AAAAGCGATCAAGGTGCTGCAGAAGGGA	13	15
FANCF_6A_5GTOT	GGAATCCCTTCTGCAGCACC			
HEK3_6A_1HIS6INS	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGTGATGATGGTGCGTGCTCAGTCTG	13	52
HEK3_6A_1HIS6INS	GGCCCAGACTGAGCACGTGA			
HEK3_6A_5GTOT	GGCCCAGACTGAGCACGTGA	TCTGCAATCACGTGCTCAGTCTG	13	10
HEK3_6A_5GTOT	GGCCCAGACTGAGCACGTGA			

HEK3_6A_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
HEK3_6A_1CTTINS	GGCCCAGACTGAGCACGTGA			
*HBB*_6B_INSALL	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14
*HBB*_6B_INSALL	GCATGGTGCACCTGACTCCTG			
*HBB*_6B_CORRECT	GCATGGTGCACCTGACTCCTG	AGACTTCTCCTCAGGAGTCAGGTGCAC	13	14
*HBB*_6B_CORRECT	GCATGGTGCACCTGACTCCTG			
*HBB*_6B_CORRECT_W_SILENT	GCATGGTGCACCTGACTCCTG	AGACTTCTCTTCAGGAGTCAGGTGCAC	13	14
*HBB*_6B_CORRECT_W_SILENT	GCATGGTGCACCTGACTCCTG			
*HEXA*_6B_INSTALL	GTACCTGAACCGTATATCCTA	AGTCAGGGCCATAGGATAGATATACGGTTC	12	14
*HEXA*_6B_CORRECT	GATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTG	10	21
*HEXA*_6B_CORRECT_W_SILENT	GATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCTTATGGCCCTGACT	9	27
*PRNP*_6C	GCAGTGGTGGGGGGCCTTGG	ATGTAGACGCCAAGGCCCCCCACC	12	12
HEK3_6E-G_1TTOG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10
HEK3_6E-G_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
RNF2_6E-G_1CTOG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
*HBB*_6E-G_4ATOT	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14
HEK3_6H_1HIS6INS	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGTGATGATGGTGCGTGCTCAGTCTG	13	52
HEK3_6H_1FLAGINS	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCCTTGTAATCCGTGCTCAGTCTG	13	58

[1408] Table 3L: FIGs. 47A-74D PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 3541-3547)	3′ EXTENSION SEQUENCE (SEQ ID NO: 3549-3556)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_ED4B_1TDEL	GGCCCAGACTGAGCACGTGA	TCTGCCATCCGTGCTCAGTCTG	13	9
HEK3_ED4B_1AINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCATCGTGCTCAGTCTG	13	11
HEK3_ED4B_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	13
HEK3_ED4C_2GTOC	GGCCCAGACTGAGCACGTGA	TCTGCCATGACGTGCTCAGTCTG	13	10
HEK3_ED4D_1FLAGINS	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCCTTGTAATCCGTGCTCAGTCTG	13	58
RNF2_ED4E_1CTOA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
EMX1_ED4F_1GTOC	GAGTCCGAGCAGAAGAAGAA	ATGGGAGCCCTTGTTCTTCTGCTCGG	13	13
*HBB*_ED4G_2TTOA	GTAACGGCAGACTTCTCCTC	ATCTGACTCCTGTGGAGAAGTCTGCC	12	14

[1409] Table 3M: FIGs. 48A-48C PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 3557-3627)	3′ EXTENSION SEQUENCE (SEQ ID NO: 3628-3698)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
VEGFA_ED5A_31	GATGTCTGCAGGCCAGATGA	CCCTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	31
VEGFA_ED5A_30	GATGTCTGCAGGCCAGATGA	CCTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	30
VEGFA_ED5A_29	GATGTCTGCAGGCCAGATGA	CTCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	29
VEGFA_ED5A_28	GATGTCTGCAGGCCAGATGA	TCTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	28
VEGFA_ED5A_27	GATGTCTGCAGGCCAGATGA	CTGACAATGTGCCATCTGGAGCACTCATCTGGCCTGCAGA	13	27

VEGFA_ED 5A_26	GATGTCTGCAGGC CAGATGA	TGACAATGTGCCATCTGGAG CACTCATCTGGCCTGCAGA	13	26
VEGFA_ED 5A_25	GATGTCTGCAGGC CAGATGA	GACAATGTGCCATCTGGAGC ACTCATCTGGCCTGCAGA	13	25
VEGFA_ED 5A_24	GATGTCTGCAGGC CAGATGA	ACAATGTGCCATCTGGAGCA CTCATCTGGCCTGCAGA	13	24
VEGFA_ED 5A_23	GATGTCTGCAGGC CAGATGA	CAATGTGCCATCTGGAGCAC TCATCTGGCCTGCAGA	13	23
VEGFA_ED 5A_22	GATGTCTGCAGGC CAGATGA	AATGTGCCATCTGGAGCACT CATCTGGCCTGCAGA	13	22
VEGFA_ED 5A_21	GATGTCTGCAGGC CAGATGA	ATGTGCCATCTGGAGCACTC ATCTGGCCTGCAGA	13	21
VEGFA_ED 5A_20	GATGTCTGCAGGC CAGATGA	TGTGCCATCTGGAGCACTCA TCTGGCCTGCAGA	13	20
VEGFA_ED 5A_19	GATGTCTGCAGGC CAGATGA	GTGCCATCTGGAGCACTCAT CTGGCCTGCAGA	13	19
VEGFA_ED 5A_18	GATGTCTGCAGGC CAGATGA	TGCCATCTGGAGCACTCATC TGGCCTGCAGA	13	18
VEGFA_ED 5A_17	GATGTCTGCAGGC CAGATGA	GCCATCTGGAGCACTCATCT GGCCTGCAGA	13	17
VEGFA_ED 5A_16	GATGTCTGCAGGC CAGATGA	CCATCTGGAGCACTCATCTG GCCTGCAGA	13	16
VEGFA_ED 5A_15	GATGTCTGCAGGC CAGATGA	CATCTGGAGCACTCATCTGG CCTGCAGA	13	15
VEGFA_ED 5A_14	GATGTCTGCAGGC CAGATGA	ATCTGGAGCACTCATCTGGC CTGCAGA	13	14
VEGFA_ED 5A_13	GATGTCTGCAGGC CAGATGA	TCTGGAGCACTCATCTGGCC TGCAGA	13	13
VEGFA_ED 5A_12	GATGTCTGCAGGC CAGATGA	CTGGAGCACTCATCTGGCCT GCAGA	13	12
VEGFA_ED 5A_11	GATGTCTGCAGGC CAGATGA	TGGAGCACTCATCTGGCCTG CAGA	13	11
VEGFA_ED 5A_10	GATGTCTGCAGGC CAGATGA	GGAGCACTCATCTGGCCTGC AGA	13	10
VEGFA_ED 5A_9	GATGTCTGCAGGC CAGATGA	GAGCACTCATCTGGCCTGCA GA	13	9
VEGFA_ED 5A_8	GATGTCTGCAGGC CAGATGA	AGCACTCATCTGGCCTGCAG A	13	8

DNMT1_ED 5B_31	GATTCCTGGTGCC AGAAACA	AGGACTAGTTCTGCCCTCCC GTCACCACTGTTTCTGGCAC CAGG	13	31
DNMT1_ED 5B_30	GATTCCTGGTGCC AGAAACA	GGACTAGTTCTGCCCTCCCG TCACCACTGTTTCTGGCACC AGG	13	30
DNMT1_ED 5B_29	GATTCCTGGTGCC AGAAACA	GACTAGTTCTGCCCTCCCGT CACCACTGTTTCTGGCACCA GG	13	29
DNMT1_ED 5B_28	GATTCCTGGTGCC AGAAACA	ACTAGTTCTGCCCTCCCGTC ACCACTGTTTCTGGCACCAG G	13	28
DNMT1_ED 5B_27	GATTCCTGGTGCC AGAAACA	CTAGTTCTGCCCTCCCGTCA CCACTGTTTCTGGCACCAGG	13	27
DNMT1_ED 5B_26	GATTCCTGGTGCC AGAAACA	TAGTTCTGCCCTCCCGTCAC CACTGTTTCTGGCACCAGG	13	26
DNMT1_ED 5B_25	GATTCCTGGTGCC AGAAACA	AGTTCTGCCCTCCCGTCACC ACTGTTTCTGGCACCAGG	13	25
DNMT1_ED 5B_24	GATTCCTGGTGCC AGAAACA	GTTCTGCCCTCCCGTCACCA CTGTTTCTGGCACCAGG	13	24
DNMT1_ED 5B_23	GATTCCTGGTGCC AGAAACA	TTCTGCCCTCCCGTCACCAC TGTTTCTGGCACCAGG	13	23
DNMT1_ED 5B_22	GATTCCTGGTGCC AGAAACA	TCTGCCCTCCCGTCACCACT GTTTCTGGCACCAGG	13	22
DNMT1_ED 5B_21	GATTCCTGGTGCC AGAAACA	CTGCCCTCCCGTCACCACTG TTTCTGGCACCAGG	13	21
DNMT1_ED 5B_20	GATTCCTGGTGCC AGAAACA	TGCCCTCCCGTCACCACTGT TTCTGGCACCAGG	13	20
DNMT1_ED 5B_19	GATTCCTGGTGCC AGAAACA	GCCCTCCCGTCACCACTGTT TCTGGCACCAGG	13	19
DNMT1_ED 5B_18	GATTCCTGGTGCC AGAAACA	CCCTCCCGTCACCACTGTTT CTGGCACCAGG	13	18
DNMT1_ED 5B_17	GATTCCTGGTGCC AGAAACA	CCTCCCGTCACCACTGTTTC TGGCACCAGG	13	17
DNMT1_ED 5B_16	GATTCCTGGTGCC AGAAACA	CTCCCGTCACCACTGTTTCT GGCACCAGG	13	16
DNMT1_ED 5B_15	GATTCCTGGTGCC AGAAACA	TCCCGTCACCACTGTTTCTG GCACCAGG	13	15

DNMT1_ED5B_14	GATTCCTGGTGCCAGAAACA	CCCGTCACCACTGTTTCTGGCACCAGG	13	14
DNMT1_ED5B_13	GATTCCTGGTGCCAGAAACA	CCGTCACCACTGTTTCTGGCACCAGG	13	13
DNMT1_ED5B_12	GATTCCTGGTGCCAGAAACA	CGTCACCACTGTTTCTGGCACCAGG	13	12
DNMT1_ED5B_11	GATTCCTGGTGCCAGAAACA	GTCACCACTGTTTCTGGCACCAGG	13	11
DNMT1_ED5B_10	GATTCCTGGTGCCAGAAACA	TCACCACTGTTTCTGGCACCAGG	13	10
DNMT1_ED5B_9	GATTCCTGGTGCCAGAAACA	CACCACTGTTTCTGGCACCAGG	13	9
DNMT1_ED5B_8	GATTCCTGGTGCCAGAAACA	ACCACTGTTTCTGGCACCAGG	13	8
RUNX1_ED5C_31	GCATTTTCAGGAGGAAGCGA	AATGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	31
RUNX1_ED5C_30	GCATTTTCAGGAGGAAGCGA	ATGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	30
RUNX1_ED5C_29	GCATTTTCAGGAGGAAGCGA	TGACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	29
RUNX1_ED5C_28	GCATTTTCAGGAGGAAGCGA	GACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	28
RUNX1_ED5C_27	GCATTTTCAGGAGGAAGCGA	ACTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	27
RUNX1_ED5C_26	GCATTTTCAGGAGGAAGCGA	CTCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	26
RUNX1_ED5C_25	GCATTTTCAGGAGGAAGCGA	TCAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	25
RUNX1_ED5C_24	GCATTTTCAGGAGGAAGCGA	CAAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	24
RUNX1_ED5C_23	GCATTTTCAGGAGGAAGCGA	AAATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	23
RUNX1_ED5C_22	GCATTTTCAGGAGGAAGCGA	AATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	22

RUNX1_ED5C_21	GCATTTTCAGGAGGAAGCGA	ATATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	21
RUNX1_ED5C_20	GCATTTTCAGGAGGAAGCGA	TATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	20
RUNX1_ED5C_19	GCATTTTCAGGAGGAAGCGA	ATGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	19
RUNX1_ED5C_18	GCATTTTCAGGAGGAAGCGA	TGCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	18
RUNX1_ED5C_17	GCATTTTCAGGAGGAAGCGA	GCTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	17
RUNX1_ED5C_16	GCATTTTCAGGAGGAAGCGA	CTGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	16
RUNX1_ED5C_15	GCATTTTCAGGAGGAAGCGA	TGTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	15
RUNX1_ED5C_14	GCATTTTCAGGAGGAAGCGA	GTCTGAAGCAATCGCTTCCTCCTGAAAAT	15	14
RUNX1_ED5C_13	GCATTTTCAGGAGGAAGCGA	TCTGAAGCAATCGCTTCCTCCTGAAAAT	15	13
RUNX1_ED5C_12	GCATTTTCAGGAGGAAGCGA	CTGAAGCAATCGCTTCCTCCTGAAAAT	15	12
RUNX1_ED5C_11	GCATTTTCAGGAGGAAGCGA	TGAAGCAATCGCTTCCTCCTGAAAAT	15	11
RUNX1_ED5C_10	GCATTTTCAGGAGGAAGCGA	GAAGCAATCGCTTCCTCCTGAAAAT	15	10
RUNX1_ED5C_9	GCATTTTCAGGAGGAAGCGA	AAGCAATCGCTTCCTCCTGAAAAT	15	9

[1410] Table 3N: FIGs. 48A-48C PEgRNA

PEGRNA	SPACER SEQUENCE	3′ EXTENSION SEQUENCE	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HEK3_ED6_5GTOA	GGCCCAGACTGAGCACGTGA (SEQ ID NO: 393)	TCTGCTATCACGTGCTCAGTCTG (SEQ ID NO: 394)	13	10

[1411] Table 3O: FIGs. 48A-48C nicking sgRNA

NICKING SGRNA	SPACER SEQUENCE

HEK3_ED6_+63	GCACATACTAGCCCCTGTCT (SEQ ID NO: 395)

[1412] Table 3P: FIGs. 50A-50B PEgRNA

PEG RNA	SPACER (SEQ ID NO: 3699-3754)	3′ EXTENSION (5′ TO 3′)(SEQ ID NO: 3755-3810)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)
HBB 3.5	GTAACGGCAGACTTCTCCAC	AGACTTCTCCTCAGGAGTCAGGTGCAC	12	14
HBB 3.7	GCATGGTGCACCTGACTCCTG	AGACTTCTCTTCAGGAGTCAGGTGCAC	13	14
HBB 5.2	GCATGGTGCACCTGACTCCTG	TAACGGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	19
HBB 5.3	GCATGGTGCACCTGACTCCTG	ACGGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	17
HBB 5.4	GCATGGTGCACCTGACTCCTG	GGCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	16
HBB 5.5	GCATGGTGCACCTGACTCCTG	GCAGACTTCTCCTCAGGAGTCAGGTGCAC	13	13
HBB 5.6	GCATGGTGCACCTGACTCCTG	GACTTCTCCTCAGGAGTCAGGTGCAC	13	12
HBB 5.7	GCATGGTGCACCTGACTCCTG	ACTTCTCCTCAGGAGTCAGGTGCAC	13	21
HBB 5.8	GCATGGTGCACCTGACTCCTG	TAACGGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	19
HBB 5.9	GCATGGTGCACCTGACTCCTG	ACGGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	17
HBB 5.10	GCATGGTGCACCTGACTCCTG	GGCAGACTTCTCCTCAGGAGTCAGGTGCA	12	16
HBB 5.11	GCATGGTGCACCTGACTCCTG	GCAGACTTCTCCTCAGGAGTCAGGTGCA	12	13
HBB 5.12	GCATGGTGCACCTGACTCCTG	GACTTCTCCTCAGGAGTCAGGTGCA	12	12
HBB 5.13	GCATGGTGCACCTGACTCCTG	ACTTCTCCTCAGGAGTCAGGTGCA	12	14
HEXAS 1	ATCCTTCCAGTCAGGGCCAT	ATATCTTATGGCCCTGACTGGAA	13	14
HEXAS 2	ATCCTTCCAGTCAGGGCCAT	TATATCTTATGGCCCTGACTGGAA	13	15
HEXAS 3	ATCCTTCCAGTCAGGGCCAT	GTATATCTTATGGCCCTGACTGGAA	13	16
HEXAS 4	ATCCTTCCAGTCAGGGCCAT	ACCGTATATCTTATGGCCCTGACTGGAA	13	19

HEX AS 5	ATCCTTCCAGTCAGGGCCAT	AACCGTATATCTTATGGCCCTGACTGGAA	13	20
HEX AS 6	ATCCTTCCAGTCAGGGCCAT	GAACCGTATATCTTATGGCCCTGACTGGAA	13	21
HEX AS 7	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAA	13	22
HEX AS 8	ATCCTTCCAGTCAGGGCCAT	ATATCTTATGGCCCTGACT	9	14
HEX AS 9	ATCCTTCCAGTCAGGGCCAT	TATATCTTATGGCCCTGACT	9	15
HEX AS 10	ATCCTTCCAGTCAGGGCCAT	GTATATCTTATGGCCCTGACT	9	16
HEX AS 11	ATCCTTCCAGTCAGGGCCAT	ACCGTATATCTTATGGCCCTGACT	9	19
HEX AS 12	ATCCTTCCAGTCAGGGCCAT	AACCGTATATCTTATGGCCCTGACT	9	20
HEX AS 13	ATCCTTCCAGTCAGGGCCAT	GAACCGTATATCTTATGGCCCTGACT	9	21
HEX AS 14	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACT	9	22
HEX AS 15	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGAC	8	22
HEX AS 16	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTG	10	22
HEX AS 17	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGG	11	22
HEX AS 18	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGA	12	22
HEX AS 19	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAA	13	22
HEX AS 20	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAAG	14	22
HEX AS 21	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCTTATGGCCCTGACTGGAAGG	15	22
HEX AS 22	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCTTATGGCCCTGACT	9	25
HEX AS 23	ATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCTTATGGCCCTGACT	9	26
HEX AS 24	ATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCTTATGGCCCTGACT	9	27
HEX AS 25	ATCCTTCCAGTCAGGGCCAT	GGTACCTGAACCGTATATCTTATGGCCCTGACT	9	28
HEX AS 26	ATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCTTATGGCCCTGACT	9	29
HEX A 5	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	21
HEX A 6	ATCCTTCCAGTCAGGGCCAT	ACCGTATATCCTATGGCCCTGACTGGAA	13	15

HEXA 7	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAAGG	15	21
HEXA 8	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGAAG	14	21
HEXA 9	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGGA	12	21
HEXA 10	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTGG	11	21
HEXA 11	ATCCTTCCAGTCAGGGCCAT	ACCTGAACCGTATATCCTATGGCCCTGACTG	10	21
HEXA 12	ATCCTTCCAGTCAGGGCCAT	AACCGTATATCCTATGGCCCTGACTGGAA	13	16
HEXA 13	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCCTATGGCCCTGACTGGAA	13	18
HEXA 14	ATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	22
HEXA 15	ATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	25
HEXA 16	ATCCTTCCAGTCAGGGCCAT	GTACCTGAACCGTATATCCTATGGCCCTGACTGGAA	13	23
HEXA 17	ATCCTTCCAGTCAGGGCCAT	AACCGTATATCCTATGGCCCTGACTG	10	16
HEXA 18	ATCCTTCCAGTCAGGGCCAT	TGAACCGTATATCCTATGGCCCTGACTG	10	18
HEXA 19	ATCCTTCCAGTCAGGGCCAT	TACCTGAACCGTATATCCTATGGCCCTGACTG	10	22
HEXA 20	ATCCTTCCAGTCAGGGCCAT	TGGTACCTGAACCGTATATCCTATGGCCCTGACTG	10	25

[1413] Table 3Q: FIGs. 50A-50B nicking sgRNA

NICKING SGRNA	SPACER SEQUENCE
*HBB*_ED7A_+72	GCCTTGATACCAACCTGCCCA (SEQ ID NO: 626)
*HEXA*_ED7B_+60	GCTGGAACTGGTCACCAAGGC(SEQ ID NO: 627)
*HEXA*_ED7B_CORRECT_WT_PE3B	GTACCTGAACCGTATATCCTA(SEQ ID NO: 628)
*HEXA*_ED7B_CORRECT_SILENT_PE3B	GTACCTGAACCGTATATCTTA(SEQ ID NO: 629)

[1414] Table 3R: FIGs. 51A-51F PEgRNA

PEGRNA	SPACER SEQUENCE (SEQ ID NO: 632-640)	3′ EXTENSION (SEQ ID NO: 641-649)	PBS LENGTH (NT)	RT TEMPLATE LENGTH (NT)

HEK3_ED8_1TTOG	GGCCCAGACTGAGCACGTGA	TCTGCCATCCCGTGCTCAGTCTG	13	10
HEK3_ED8_3ATOC	GGCCCAGACTGAGCACGTGA	TCTGCCAGCACGTGCTCAGTCTG	13	10
HEK3_ED8_3ATOT	GGCCCAGACTGAGCACGTGA	TCTGCCAACACGTGCTCAGTCTG	13	10
HEK3_ED8_3ATOT_5-6GGTOTT	GGCCCAGACTGAGCACGTGA	TGGAGGAAGCAGGGCTTCCTTTCCTCTGAAAACACGTGCTCAGTCTG	13	34
HEK3_ED8_1CTTINS	GGCCCAGACTGAGCACGTGA	TCTGCCATCAAAGCGTGCTCAGTCTG	13	10
RNF2_ED8_1CTOA	GTCATCTTAGTCATTACCTG	AACGAACACCTCATGTAATGACTAAGATG	15	14
RNF2_ED8_1CTOG	GTCATCTTAGTCATTACCTG	AACGAACACCTCACGTAATGACTAAGATG	15	14
RNF2_ED8_1GTAINS	GTCATCTTAGTCATTACCTG	AACGAACACCTCAGTACGTAATGACTAAGATG	15	17
*HBB*_ED8_4ATOT	GCATGGTGCACCTGACTCCTG	AGACTTCTCCACAGGAGTCAGGTGCAC	13	14

[1415] Table 4: Sequences of primers used for mammalian cell genomic DNA amplification and HTS[181].

DESCRIPTION	SEQUENCE (SEQ ID NOS: 3811-3863)
HEK3 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNATGTGGGCTGCCTAGAAAGG
HEK3 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCCAAACTTGTCAACC
RNF2 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACGTCTCATATGCCCCTTGG
RNF2 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTAGGAATTTTGGTGGGACA
HEK4 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAACCCAGGTAGCCAGAGAC
HEK4 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTTCAACCCGAACGGAG
EMX1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGCTCAGCCTGAGTGTTGA
EMX1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGTGGGTTTGTGGTTGC
FANCF FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATTGCAGAGAGGCGTATCA
FANCF REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGTCCCAGGTGCTGAC
HBB FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNAGGGTTGGCCAATCTACTCCC
HBB REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTTCTCTGTCTCCACATGCC

PRNP FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTCAG TGGAACAAGCCGAGT
PRNP REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGGTTGGGGT AACGGTG
HEXA FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCATAC AGGTGTGGCGAGAGG
HEXA REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCTCCTTTG GTTAGCA
RUNX1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCACA AACAAGACAGGGAACTG
RUNX1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTAGATGTAGGGCTA GAGGGGTG
VEGFA FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNACTTG GTGCCAAATTCTTCTCC
VEGFA REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGAGGGAATG GGCTTTGGA
DNMT FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCACAA CAGCTTCATGTCAGCC
DNMT REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTACGTTAATGTTTC CTGATGGTCC
HEK3 OFF-TARGET SITE 1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCCC TGTTGACCTGGAGAA
HEK3 OFF-TARGET SITE 1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGTACTTGCC CTGACCA
HEK3 OFF-TARGET SITE 2 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTGGT GTTGACAGGGAGCAA
HEK3 OFF-TARGET SITE 2 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGATGTGGGC AGAAGGG
HEK3 OFF-TARGET SITE 3 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTGAGA GGGAACAGAAGGGCT
HEK3 OFF-TARGET SITE 3 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCAAAGGCCC AAGAACCT
HEK3 OFF-TARGET SITE 4 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTCCTA GCACTTTGGAAGGTCG
HEK3 OFF-TARGET SITE 4 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCATCTTAATCT GCTCAGCC
HEK4 OFF-TARGET SITE 1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGCAT GGCTTCTGAGACTCA
HEK4 OFF-TARGET SITE 1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCCTTGCAC TCCCTGTCTTT
HEK4 OFF-TARGET SITE 2 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTGG CAATGGAGGCATTGG
HEK4 OFF-TARGET SITE 2 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGAGGCTGCC CATGAGAG
HEK4 OFF-TARGET SITE 3 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGGTCT GAGGCTCGAATCCTG
HEK4 OFF-TARGET SITE 3 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGGCCTCCAT ATCCCTG

HEK4 OFF-TARGET SITE 4 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNTTTCC ACCAGAACTCAGCCC
HEK4 OFF-TARGET SITE 4 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCGGTTCCTCC ACAACAC
EMX1 OFF-TARGET SITE 1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTGGG GAGATTTGCATCTGTGGAGG
EMX1 OFF-TARGET SITE 1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTATACCATC TTGGGGTTACAG
EMX1 OFF-TARGET SITE 2 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAATG TGCTTCAACCCATCACGGC
EMX1 OFF-TARGET SITE 2 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGAATTTGTG ATGGATGCAGTCTG
EMX1 OFF-TARGET SITE 3 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGAGAA GGAGGTGCAGGAGCTAGAC
EMX1 OFF-TARGET SITE 3 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCATCCCGACCTTC ATCCCTCCTGG
EMX1 OFF-TARGET SITE 4 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGTAGT TCTGACATTCCTCCTGAGGG
EMX1 OFF-TARGET SITE 4 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAACAAGGTG CAGATACAGCA
FANCF OFF-TARGET SITE 1 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNGCGGG CAGTGGCGTCTTAGTCG
FANCF OFF-TARGET SITE 1 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGGGTTTGGT TGGCTGCTC
FANCF OFF-TARGET SITE 2 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCTCCT TGCCGCCCAGCCGGTC
FANCF OFF-TARGET SITE 2 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGGGGAAGA GGCGAGGACAC
FANCF OFF-TARGET SITE 3 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCCAGT GTTTCCCATCCCCAACAC
FANCF OFF-TARGET SITE 3 REV	TGGAGTTCAGACGTGTGCTCTTCCGATCTGAATGGATCCCCC CCTAGAGCTC

FANCF OFF-TARGET SITE 4 FWD	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNCAGGC CCACAGGTCCTTCTGGA

[1416] Table 5: Sequences of 100-mer single-stranded DNA oligonucleotide donor templates used in HDR experiments and in the creation of the *HBB* E6V HEK293T cell line. Oligonucleotides are 100-103 nt in length with homology arms centered around the site of the edit. Oligonucleotides were from Integrated DNA Technologies, purified by PAGE.

HEK3 +3 A TO T	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAG ACTGAGCACGTGTTGGCAGAGGAAAGGAAGCCCTGCTTC CTCCAGAGGGCGTCGCAGGAC (SEQ ID NO: 717)
HEK3 +3 A TO T, +5,6 GG TO TT	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAG ACTGAGCACGTGTTTTCAGAGGAAAGGAAGCCCTGCTTC CTCCAGAGGGCGTCGCAGGAC (SEQ ID NO: 718)
HEK3 +1 T TO G	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAG ACTGAGCACGGGATGGCAGAGGAAAGGAAGCCCTGCTTC CTCCAGAGGGCGTCGCAGGAC (SEQ ID NO: 719)
HEK3 +3 A TO C	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAG ACTGAGCACGTGCTGGCAGAGGAAAGGAAGCCCTGCTTC CTCCAGAGGGCGTCGCAGGAC (SEQ ID NO: 720)
HEK3 +1 CTT INSERTION	GCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAG ACTGAGCACGCTTTGATGGCAGAGGAAAGGAAGCCCTGC TTCCTCCAGAGGGCGTCGCAGGAC (SEQ ID NO: 721)
RNF2 +1 C TO A	CCCAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTA GTCATTACATGAGGTGTTCGTTGTAACTCATATAAACTGAG TTCCCATGTTTTGCTTAA (SEQ ID NO: 722)
RNF2 +1 C TO G	CCCAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTA GTCATTACGTGAGGTGTTCGTTGTAACTCATATAAACTGAG TTCCCATGTTTTGCTTAA (SEQ ID NO: 723)
RNF2 +1 GTA INSERTION	CAGTTTACACGTCTCATATGCCCCTTGGCAGTCATCTTAGT CATTACGTACTGAGGTGTTCGTTGTAACTCATATAAACTGA GTTCCCATGTTTTGCTTA (SEQ ID NO: 724)
HBB E6V INSTALLATION (ALSO USED FOR CREATION OF THE *HBB* E6V HEK293T CELL LINE)	ACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGG CAGACTTCTCCACAGGAGTCAGATGCACCATGGTGTCTGT TTGAGGTTGCTAGTGAACAC (SEQ ID NO: 725)
HBB E6V CORRECTION PROTOSPACER A	ACTTCATCCACGTTCACCTTGCCCCACAGGGCAGTAACGG CAGACTTCTCCTCAGGAGTCAGGTGCACCATGGTGTCTGT TTGAGGTTGCTAGTGAACAC (SEQ ID NO: 726)
HBB E6V CORRECTION PROTOSPACER B	GTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCT GACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGC AAGGTGAACGTGGATGAAGT (SEQ ID NO: 727)
HBB E6V CORRECTION PROTOSPACER B,	GTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCT GACTCCTGATGAGAAGTCTGCCGTTACTGCCCTGTGGGGC AAGGTGAACGTGGATGAAGT (SEQ ID NO: 728)

SILENT PAM MUTATION	
PRNP G127V	CACATGGCTGGTGCTGCAGCAGCTGGGGCAGTGGTGGGG GGCCTTGGCGTCTACATGCTGGGAAGTGCCATGAGCAGGC CCATCATACATTTCGGCAGTG (SEQ ID NO: 729)

[1417] Additional Sequences

[1418] Sequences of yeast dual fluorescent reporter plasmids used herein

p425-GFP_stop_mCherry:

ATGTCTAAAGGTGAAGAATTATTCACTGGTGTTGTCCCAATTTTGGTTGAATTAGAT
GGTGATGTTAATGGTCACAAATTTTCTGTCTCCGGTGAAGGTGAAGGTGATGCTAC
TTACGGTAAATTGACCTTAAAATTTATTTGTACTACTGGTAAATTGCCAGTTCCATG
GCCAACCTTAGTCACTACTTTCGGTTATGGTGTTCAATGTTTTGCTAGATACCCAGA
TCATATGAAACAACATGACTTTTTCAAGTCTGCCATGCCAGAAGGTTATGTTCAAG
AAAGAACTATTTTTTTCAAAGATGACGGTAACTACAAGACCAGAGCTGAAGTCAA
GTTTGAAGGTGATACCTTAGTTAATAGAATCGAATTAAAAGGTATTGATTTTAAAGA
AGATGGTAACATTTTAGGTCACAAATTGGAATACAACTATAACTCTCACAATGTTTA
CATCATGGCTGACAAACAAAAGAATGGTATCAAAGTTAACTTCAAAATTAGACAC
AACATTGAAGATGGTTCTGTTCAATTAGCTGACCATTATCAACAAAATACTCCAATT
GGTGATGGTCCAGTCTTGTTACCAGACAACCATTACTTATCCACTCAATCTGCCTTA
TCCAAAGATCCAAACGAAAAGAGAGACCACATGGTCTTGTTAGAATTTGTTACTG
CTGCTGGTATTACCCATGGTATGGATGAATTGTACAAAGCTAGCAACCTGGGTCAA
TCCTTGG**GGCCCAGACTGAGCACGTGATGG**CAGAGCACAGGAGACGTC*ATGGT*
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GGGATATTTTGTCACCACAATTTATGTATGGTTCAAAAGCTTATGTTAAACATCCAGCTGA
TATTCCAGATTATTTAAAATTGTCATTTCCAGAAGGTTTTAAATGGGAAAGAGTTATGAATT
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ACTATGGGTTGGGAAGCTTCATCAGAAAGAATGTATCCAGAAGATGGTGCTTTAAAAGG
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p425-GFP_+1fs_mCherry:

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KEY:
GFP open reading frame
Linker containing stop codon +1 frameshift, or -1 frameshift
mCherry open reading frame
Plasmid backbone (containing the GPD promoter, Leu2 marker, and AmpR)
Protospacer (underlined)
PAM (boldfaced)

[1419] DNA sequences of mammalian prime editor plasmids and example PEgRNA plasmid

pCMV-PE1:

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GGAGAGCCGCCACC (SEQ ID NO: 734)

N-terminal NLS + Cas9 H840A
Flexible linker

M-MLV reverse transcriptase + C-terminal NLS
Plasmid backbone (containing CMV promoter and AmpR)

pU6-HEK3_PEgRNA_CTTins:

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TACCAAGGTCGGGCAGGAA (SEQ ID NO: 735)

U6 Promoter sequence
Spacer sequence

sgRNA scaffold
3′ extension (contains PBS and RT template)
Backbone (contains AmpR)

pLenti-hSyn-N-PE2-NpuN-P2A-GFP-KASH_U6-DNMT1-PEgRNA:

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U6promoter
PEgRNA
hSynpromoter
N-termPE2
N-termNpu
P2A-GFP-KASH

pLenti-hSyn-C-PE2-NpuC:

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GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAG
CGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGG
AACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCT
GTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCG
GAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGG
CCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCG
CCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGT
GAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC
GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGC
AACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTT
CCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCT
ATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCTGGA
GCTGCAAGCTTAATGTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATG
AGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAA
GTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATTG
GACGAACCACTGAATTGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATAC
ATAAACGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGG
GAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCC
GTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAA
ATCTCTAGCAGTGGCGCCCGAACAGGGACTTGAAAGCGAAAGGGAAACCAGAGGAGC
TCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGC
GACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAGAGATGGGT
GCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGCGATGGGAAAAAATTCGGTT
AAGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAG
CTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACAAAT
ACTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATA
ATACAGTAGCAACCCTCTATTGTGTGCATCAAAGGATAGAGATAAAAGACACCAAGGA
AGCTTTAGACAAGATAGAGGAAGAGCAAAACAAAAGTAAGACCACCGCACAGCAAGC
GGCCGCTGATCTTCAGACCTGGAGGAGGAGATATGAGGGACAATTGGAGAAGTGAATT
ATATAAATATAAAGTAGTAAAAATTGAACCATTAGGAGTAGCACCCACCAAGGCAAAGA
GAAGAGTGGTGCAGAGAGAAAAAAGAGCAGTGGGAATAGGAGCTTTGTTCCTTGGGT
TCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGG

CCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAG
GCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGA
ATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTGGGGATTTGGGGTTGCT
CTGGAAAACTCATTTGCACCACTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCT
CTGGAACAGATTTGGAATCACACGACCTGGATGGAGTGGGACAGAGAAATTAACAATT
ACACAAGCTTAATACACTCCTTAATTGAAGAATCGCAAAACCAGCAAGAAAAGAATGA
ACAAGAATTATTGGAATTAGATAAATGGGCAAGTTTGTGGAATTGGTTTAACA (SEQ
ID NO: 738)
U6promoter
sgRNA

[1420] Amino acid sequences of Maloney murine leukemia virus reverse transcriptase (M-MLV RT) variants used herein.

PE1 M-MLV RT:

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTP
VSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLR
EVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR
DPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET
VMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAY
QEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPV
AAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMT
HYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPD
ADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKM
AEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLS
IIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 739)

M3 M-MLV RT (D200N, T330P, L603W) (see Baranauskas et al.[182]):

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTP
VSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLR
EVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR
DPEMGISGQLTWTRLPQGFKNSPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET
VMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQQKAY
QEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPV
AAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMT
HYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPD
ADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKM
AEGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKRL
SIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO:
740)

PE2 M-MLV RT (D200N, T306K, W313F, T330P, L603W):

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTP
VSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLR

EVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR
DPEMGISGQLTWTRLPQGFKNSPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET
VMGQPTPKTPRQLREFLG**K**AGFCRL**F**IPGFAEMAAPLYPLTK**P**GTLFNWGPDQQKAY
QEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPV
AAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMT
HYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPD
ADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKM
AEGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKRL
SIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 741)

M3-deadRT M-MLV RT:

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTP
VSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVK**L**PGTNDY**S**PVQDLR
EVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR
DPEMGISGQLTWTRLPQGFKNSPTLF**N**EALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKET
VMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTK**P**GTLFNWGPDQQKAY
QEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPV
AAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMT
HYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPD
ADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKM
AEGKKLNVYTDSRYAFATAHIHGEIYRRRG**W**LTSEGKEIKNKDEILALLKALFLPKRL
SIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP (SEQ ID NO: 742)

References for Example 12

[1421] Each of the following references are cited in Example 12, each of which are incorporated herein by reference.

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EXAMPLE 13 - CELL DATA RECORDING AND LINEAGE TRACING BY PE

Background

[1422] Genome modification can be used to study and record cellular processes and development. Linking cellular events, like cell division or signaling cascade activation, to DNA sequence modifications stores cellular histories as interpretable DNA sequence changes that would describe whether specific cellular events had taken place. DNA editing is necessary for these applications because DNA is faithfully passed from one cell to the next in a way that RNA and proteins are not. Information relating to cellular state and lineage are in general lost when modifications are made to short-lived protein and RNA molecules. Recording cellular events within a single cell is a powerful way to understand how disease states are initiated, maintained, and changed relative to healthy controls. The ability to probe these questions has implications for understanding the development of cancer, neurological disease, and a host of other important problems in human health. prime editing (PE) provides a system for creating targeted and sequence-specified genomic insertions, deletions, or mutations. Repeated modification of a DNA target that can be sequenced by targeted amplicon sequencing and/or RNA sequencing (which is of particular value for single cell recording experiments) can be used to record a host of important biological processes, including activation of signaling cascades, metabolic states, and cellular differentiation programs. Connecting internal and external cellular signals to sequence modifications in the genome is, in theory, possible for any signal for which a signal responsive promoter exists. It is believed that PE enables a greatly expanded toolkit for probing the cellular lineage and the signaling histories of eukaryotic and prokaryotic cells in both cultured conditions and *in vivo*.

[1423] Previous standards

[1424] Targeted sequence insertion, deletion, or mutation can be used to study a number of important biological questions including lineage tracing and recording of cellular stimuli. The current toolkit for generating these signatures in the genome is limited. Mutagenesis of target loci has been developed to date using both DNA-nucleases and base editors.

[1425] CRISPR/Cas9 nuclease cutting of target sequences generates stochastic sequence changes to generate a large number of insertion or deletion (indel) products. The large number of sequence outcomes that arise from Cas9 cutting allows for clear determination of sequences that have been cut by the nuclease. The ability to distinguish cut versus non-cut sequences has been used in two predominant ways.

[1426] First, the expression of the Cas9 nuclease and/or its single guide RNA (sgRNA) have been connected to cellular signals. Additionally, whether that signal has occurred based on sequence modifications to the Cas9 targeted genomic locus has been recorded. However, this approach is limited because each signal requires a unique target locus, which makes tracking the relative timing of multiple signals difficult to interpret. Another limitation to this approach is that multiple target loci are desired for a specific sgRNA because generation of an indel often severely hinders additional mutagenesis of the target locus; this often means that pre-engineered target loci are integrated into cells for editing instead of direct mutagenesis of endogenous loci.

[1427] Second, Cas9 indels have been used to track cellular lineages. As described herein, the large number of possible indel states generated by Cas9 nuclease activity allows for the generation of cellular developmental trees that suggest which cells have arisen from one another across time. This approach is a powerful way to understand how cells arise from one another and has been used to help identify unique cell states and types across developmental time by performing RNA sequencing on selected cellular pools. This approach cannot independently report on cellular signaling events, their order, and may have biases when reporting on pre-cursor versus terminally differentiated cell states.

[1428] Cas9 nuclease mediated lineage tracing and signal recording are powerful techniques that come with some important caveats. Signal recording with Cas9 nuclease is often very technically challenging. Cas9 cutting exhausts the target locus (repeated cutting is difficult once an indel is generated) making it difficult to record long term stimuli at the single cell level. The kinetics of Cas9 cutting can be tuned to enable longer term recording events though the ability to integrate the order, intensity, and duration of multiple stimuli remains a

very challenging technical problem that may not be achievable with this tool. Cas9 lineage tracing experiments have been incredibly powerful but suffer from minor technical challenges of sequence collapse due to simultaneous Cas9 cuts at a target locus. These lineage-tracing experiments require editing of pre-designed target loci, limiting the flexibility of this approach.

[1429] DNA base editing has also been used to track cellular signaling events. Base editing is not well suited for lineage tracing due to the low number of outcome states generated by an editing event relative to the number of states generated by Cas9 indels; however, the pre-defined nature of the sequence modifications made by a base editor are particularly useful for tracking internal and external cellular stimuli. As described herein, either base editor or sgRNA expression can be connected to a particular biological or chemical stimulus. Base editing activity has been used to track a large number of individual stimuli in both mammalian and bacterial cells. This approach has also been used to track consecutive stimuli where a first editing event was necessary before a second edit could take place.

[1430] Base editing signal recording is an important first step for the field, but it has a number of limitations. One such limitation is that base editing exhausts its target after editing, limiting the dynamic range of the technique. This means that using endogenous targets for recording events is often difficult and limited to recording bulk activities instead of activity at the single cell level. An alternative to this is the introduction of a pre-designed repetitive recording locus though this has not been performed to date. There are also issues with two-signal recording. These two signal-recording experiments only report on the presence of the second stimulus after a first; it does not report which stimulus happened first or how long the stimuli were present. This fundamentally limits the biological understanding gleaned from the experiment.

[1431] It has been proposed that PE lineage tracing can do both lineage tracing and cellular signaling recording by modifying genomic target sequences as well as integrated pre-designed sequences. PE uses a synthetic fusion protein comprising a Cas9 nickase fragment (often the SpCas9 H840A variant) and a reverse transcriptase (RT) domain, along with an engineered prime editing guide RNA (PEgRNA). Together, these components target a specific genomic sequence and install a pre-determined edit. Since the PEgRNA specifies both the target genomic sequence and the editing outcome, highly specific and controlled genome modification can be achieved simultaneously using multiple PEgRNAs within the same cell. Accessible genome modifications include all single nucleotide substitution, small

to medium size sequence insertions, and small to medium size sequence deletions. The versatility of this genome editing technology should enable temporally coupled, signal-specific recording within cells.

[1432] Utility of PE lineage and cell signaling recording

[1433] Recording cellular signaling can be accomplished in a number of ways. One important first application of this approach is to connect DNA modification events to cell cycle associated signals like the expression of cyclins, CDKs, or other proteins specific to phases of the cellular life span one could generate a cellular clock. A cellular clock allows researchers to understand the order of various signals being received and processed by individual cells. A molecular clock would also enable the determination of long-term signaling versus short-term bursts of signaling. Using prime editing components that can only edit once a cell cycle could also lead to a molecular clock. If editing can only proceed once a cell cycle without continued DNA modification (perhaps by not nicking the non-edited DNA strand) one could imagine a system that only can be edited by a second targeting PEsgRNA in a subsequent cell division. PE is particularly useful as a cellular clock as it can repeatedly insert, delete, or mutate loci in a predetermined way with insertions being particularly valuable as repeated, regular insertions can be made at any target genomic locus.

[1434] Another important application related to recording cellular signals is the parallel recording of a large number of cellular inputs. Linking cellular signaling events to DNA modification enables recording of whether such a signaling event has occurred. Similar to Cas9 nuclease-based or base editing-based recording systems, recording of cellular events can be tethered to gRNA or editor expression. Unlike these other approaches, lineage prime editing should be able to record the order, intensity, and duration of signaling events without requiring strict sequence motifs for ordered editing. Indeed, lineage prime editing should be able to integrate the cellular counter described above with signal specific insertions, deletions, or mutations to study the order, intensity, and duration of biological signals. Due to the programmable nature of prime editing, this approach can be achieved at genomic loci pre-existing in the target cell of interest (whether this is in bacteria, mice, rats, monkeys, pigs, humans, zebrafish, *C. elegans*, etc.). It is important to note as well that prime editing recording installs barcodes in a guide RNA dependent manner, the number of inputs is limited to the number of signals for which there are reliable signal specific guide RNA expression cassettes (which should be very high due to the ability to tether these expressions

to the activity of RNA Pol II promoters). The number of recordable signals scales linearly with the number of PEgRNAs needed.

[1435] PE can also be used to trace cellular lineages. Repeated sequence modification can be used to generate unique cellular barcodes to track individual cells. The arrays of barcodes, their order, and size can all be used to infer cellular lineages in a way that can be complementary to the large number of indel states generated by Cas9 nuclease.

[1436] Prime editing methodology for repeated sequence modification

[1437] A number of distinct modalities for repeated sequence modification using prime editing (PE) were envisioned: DNA mutagenesis; sequence deletion; and, sequence insertion. Notably, these applications can be used on either pre-existing genomic DNA targets or on pre-designed DNA sequences that researchers integrate into target cells. These techniques of serial sequence modification have value for recording information and for designed or stochastic modification of target loci in a continuous manner. Serial targeted locus modification may be particularly useful for generating libraries of variants in various hosts.

[1438] Repeated sequence mutation can be used to alter either genomic DNA or pre-designed integrated DNA sequences in an iterative manner to report on cellular signaling events. In this paradigm, mutations installed by PE gRNA activity will correspond to the presence of a cellular signal. These point mutations could install PAM motifs necessary for serial editing events, as well as point mutations that correspond to the presence of specific signals. This system would require gRNA design prior to use as each successive guide RNA will use a novel protospacer; however, it could be especially powerful for examining individual or small numbers of stimuli of particular interest. Installation of these mutations could be dependent on individual biological stimuli or could be connected to consistent cellular processes that would mark cellular time. The sequences below correspond to SEQ ID NOs: 743, 744, 744, and 745.

Target genomic DNA sequence	CGTATCGGTAACTGATCCGATGGAAAGCCAGTTCAGAACCG
Target sequence post edit #1 (install G and T)	CGTATCGGTAACTGATCCGATGGAATGCCAGGTCAGAACCG
Target sequence post edit #1 (use AGG PAM)	CGTATCGGTAACTGATCCGATGGAATGCCAGGTCAGAACCG
Target sequence post edit #2 (install G and A)	CGTATCGGTAACTGATCCGATGGAATGCCAGGTCAAAACGG

[1439] Another similar PE guide RNA-intensive method is the repeated deletion of target sequences. Removal of individual sequences from a target locus would allow for the ability to

reconstruct signaling events through the loss of DNA motifs. Design of PEgRNAs that delete successive sequences would enable the tracking of consecutive signals. This would allow researchers to identify instances where one signal followed another, which would allow researchers to probe which signaling events happen in which order. Such a system has been tested using CAMERA; however, this required pre-selection of particular loci with unique sequence requirements. Successive sequence deletion using PE would allow for the paralleled recording of pairwise events in individual cells as no specific sequence determinants are required. This would allow researchers the ability to probe pairwise signaling events in a multiplexed manner inside any target cell of interest. The sequences below correspond to SEQ ID NOs: 746, 747, and 748.

Example target DNA sequence	CGTATCGGTAACTGATCCGATGGAAAGCCAGTTCAGAACCG
Target sequence post edit #1 (delete AAA)	CGTATCGGTAACTGATCCGATGGGCCAGTTCAGAACCG

[1440] Target sequence post edit #2 (delete GCC) CGTATCGGTAACTGATCCGATGGAGTTCAGAACCG

[1441] Sequence insertion is a third approach for the tracking cellular signaling events. Some variants of this strategy are less PEgRNA-dependent than mutagenesis or deletion. A number of different insertion strategies exist- insertion of short sequences, insertion of protospacers, insertion of a protospacer and a barcode, insertion of novel homology sequences, and insertion of homology sequences with a barcode.

[1442] Insertion of short repetitive sequences is a way to incrementally increase the size of a target sequence to measure the passage of time in a cell. In this system insertion of 5 or more nucleotides of repetitive sequence can cause repeat expansion in connection to either the passage of time or the continued presence of a pre-determined stimulus. The locus-agnostic nature of lineage PE again enables paralleled tracking of multiple unique sequence expansions in connection with discrete biological signals. This should enable measuring of the intensity of multiple biological signals across cellular time in individual cells. The sequences below correspond to SEQ ID NOs: 749, 750, and 751.

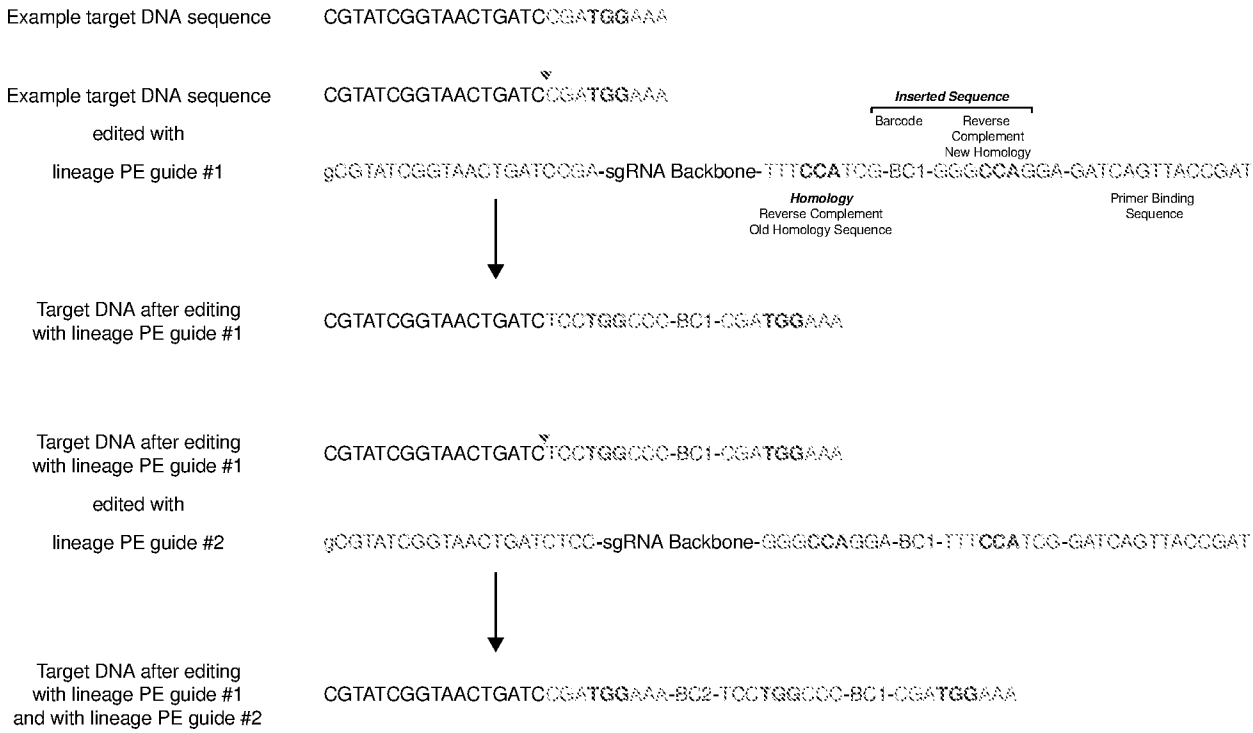
Example target DNA sequence	genomic sequence-CGTATCGTATCGTATCGTATTGG-genomic sequence
Target sequence post edit #1 (install CGTAT)	genomic sequence-CGTATCGTATCGTATCGTATCGTATTGG-genomic sequence

[1443] Target sequence post edit #2 (install CGTAT) genomic sequence-CGTATCGTATCGTATCGTATCGTATCGTATTGG-genomic sequence

[1444] Insertion of different short sequences would require a handful of PEgRNAs in a manner similar to the deletional space. The number of signals being recorded and the size of the inserted sequence would determine the number of combinations of PEgRNAs needed.

One challenge with recording multiple sequences in this system would be the different efficiencies of each PEgRNA at inserting its cargo sequence.

[1445] Insertion of protospacers as indicators of cellular signals is enticing though technical challenges may infringe on the efficiency of this approach. Single PEgRNA systems will be challenging to use because PEgRNA cassettes would be substrates for themselves, causing insertions into the PEgRNA that compromise the efficiency and fidelity of successive edits. This same problem persists for two or three PEgRNA systems, as each guide is a substrate for another, enabling insertion of other sequences into the guide cassette itself, which could lead to inappropriate insertion of protospacer sequences in connection with the wrong signal. These protospacer insertion systems are also difficult to imagine with the inclusion of barcode sequences. Single PEgRNA barcode systems would simply write over the barcodes used, removing the data stored in the first edit. Multiple guide systems again suffer from insertions into other PEgRNA expression constructs, limiting its utility (especially *in vivo*). The sequences below correspond to SEQ ID NOs: 752, 752, 753, 754, 754, 755, and 756.



[1446]

[1447] Insertion of homology sequences *(i.e.,* sequences 3′ of the Cas9 nick location), and especially homology sequences with associated barcodes, appear to be particularly useful lineage PE strategies. This system avoids the issues associated with protospacer insertion by ensuring that successive rounds of editing result in the insertion of a barcode from a PEgRNA cassette that cannot be modified by other PEgRNA editing events in the same cell. The

barcoding system is valuable as multiple barcodes can be associated with a given stimulus. This system preserves the majority of the target protospacer but alters the seed sequence, PAM, and downstream adjacent nucleotides. This enables multiple signals to be connected to one editing locus without significant re-designing of the PEgRNAs being used. This strategy would enable multiplexed barcode insertion in response to a large number of cellular stimuli (either internal or external) at a single locus. It could enable recording of intensity, duration, and order of as many signals as there exist unique barcodes (which can be designed with multiple N nucleotides to generate 4^N possible barcodes; i.e. a 5-nt barcode would enable recording of 4^5 or 1024 unique signals at once). This system could be used both *in vitro* and *in vivo*.

References cited in Example 13

[1448] Each of the following references are incorporated herein by reference.

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2. Whole-organism lineage tracing by combinatorial and cumulative genome editing.Mckenna et al. Science. 2016 Jul 29;353(6298):aaf7907. doi10.1126/science.aaf7907. Epub 2016 May 26.

3. Molecular recording of mammalian embryogenesis. Chan et al. 2019. *Nature*. Jun;570(7759):77-82. doi: 10.1038/s41586-019-1184-5. Epub 2019 May 13.

EXAMPLE 14 – MODULATING BIOMOLECULE ACTIVITY AND/OR LOCALIZATION BY PE

[1449] The subcellular localization and modification states of biomolecules regulate their activities. Specific biological functions like transcriptional control, cellular metabolism, and signal transduction cascades are all carefully orchestrated in particular locations within the cell. As such, modulating the cellular localization and modification states of proteins represents a potential therapeutic strategy for the treatment of disease. Some existing therapeutics have been developed to alter the localization of target proteins. For example, farnesylation inhibitors are designed to prevent lipidation and membrane targeting of important oncogenic proteins like KRAS. Similarly, small-molecule-induced ubiquitination of target proteins directs them to the proteasome for degradation. The ability to traffic proteins to these and other unique cellular compartments provides an opportunity to alter a number of biological processes. It is proposed herein that the use of prime editing (PE) to

install genetically encoded handles for altering the modification state and the subcellular trafficking of biomolecules with a genetically encoded signal (e.g. proteins, lipids, sugars, and nucleic acids) for the purposes of therapeutics.

[1450] PE is a genome editing technology that enables the installation, deletion, or replacement of short DNA sequences into any genomic locus targetable with a Cas9 enzyme. Using this technology, one could in principle install or remove important DNA, RNA, or protein coding sequences that change the activities of these important biomolecules. More specifically, prime editing could be used to install motifs, or signals, that change the localization or modification properties of biomolecules. Some examples include modification to: protein amino acid sequences; motifs for post translational modifications; RNA motifs that change folding or localization; and, installation of DNA sequences that change the local chromatin state or architecture of the surrounding DNA.

[1451] One target biomolecule for PE-mediated modification is DNA. Modifications to DNA could be made to install a number of DNA sequences that change the accessibility of the target locus. Chromatin accessibility controls gene transcriptional output. Installation of marks to recruit chromatin compacting enzymes should decrease the transcriptional output of neighboring genes, while installation of sequences associated with chromatin opening should make regions more accessible and in turn increase transcription. Installation of more complex sequence motifs that mirror native regulatory sequences should provide more nuanced and biologically sensitive control than the currently available dCas9 fusions to different epigenetic reader, writer, or eraser enzymes–tools that typically install large numbers of a single type of mark that may not have a particular biological antecedent. Installation of sequences that will bring two loci into close proximity, or bring loci into contact with the nuclear membrane, should also alter the transcriptional output of those loci as has been demonstrated in the burgeoning field of 3-D genomic architecture.

[1452] Modifications to RNAs can also be made to alter their activity by changing their cellular localization, interacting partners, structural dynamics, or thermodynamics of folding. Installation of motifs that will cause translational pausing or frameshifting could change the abundance of mRNA species through various mRNA processing mechanisms. Modifying consensus splice sequences would also alter the abundance and prevalence of different RNA species. Changing the relative ratio of different splice isoforms would predictably lead to a change in the ratio of protein translation products, and this could be used to alter many biological pathways. For instance, shifting the balance of mitochondrial versus nuclear DNA

repair proteins would alter the resilience of different cancers to chemotherapeutic reagents. Furthermore, RNAs could be modified with sequences that enable binding to novel protein targets. A number of RNA aptamers have been developed that bind with high affinity to cellular proteins. Installation of one of these aptamers could be used to either sequester different RNA species through binding to a protein target that will prevent their translation, biological activity, or to bring RNA species to specific subcellular compartments. Biomolecule degradation is another class of localization modification. For example, RNA methylation is used to regulate RNAs within the cell. Consensus motifs for methylation could be introduced into target RNA coding sequences with PE. RNAs could also be modified to include sequences that direct nonsense mediated decay machinery or other nucleic acid metabolism pathways to degrade the target RNA species would change the pool of RNAs in a cell. Additionally, RNA species could be modified to alter their aggregation state. Sequences could be installed on single RNAs of interest or multiple RNAs to generate RNA tangles that would render them ineffective substrates for translation or signaling.

[1453] Modifications to proteins via post-translational modification (PTM) also represent an important class of biomolecule manipulation that can be carried out with PE. As with RNA species, changing the abundance of proteins in a cell is an important capability of PE. Editing can be done to install stop codons in an open reading frame–this will eliminate full-length product from being produced by the edited DNA sequence. Alternatively, peptide motifs can be installed that cause the rate of protein degradation to be altered for a target protein. Installation of degradation tags into a gene body could be used to alter the abundance of a protein in a cell. Moreover, introduction of degrons that are induced by small molecules could enable temporal control over protein degradation. This could have important implications for both research and therapeutics as researchers could readily assess whether small molecule-mediated therapeutic protein degradation of a given target was a viable therapeutic strategy. Protein motifs could also be installed to change the subcellular localization of a protein. Amino acid motifs can be installed to preferentially traffic proteins to a number of subcellular compartments including the nucleus, mitochondria, cell membrane, peroxisome, lysosome, proteasome, exosome, and others.

[1454] Installing or destroying motifs modified by PTM machinery can alter protein post-translational modifications. Phosphorylation, ubiquitylation, glycosylation, lipidation (e.g. farnesylation, myristoylation, palmitoylation, prenylation, GPI anchors), hydroxylation, methylation, acetylation, crotonylation, SUMOylation, disulfide bond formations, side chain

bond cleavage events, polypeptide backbone cleavage events (proteolysis), and a number of other protein PTMs have been identified. These PTMs change protein function, often by changing subcellular localization. Indeed, kinases often activate downstream signaling cascades via phosphorylation events. Removal of the target phosphosite would prevent signal transduction. The ability to site-specifically ablate or install any PTM motif while retaining full-length protein expression would be an important advance for both basic research and therapeutics. The sequence installation scope and target window of PE make it well suited for broad PTM modification space.

[1455] Removal of lipidation sites should prevent the trafficking of proteins to cell membranes. A major limitation to current therapeutics that target post-translational modification processes is their specificity. Farnesyl transferase inhibitors have been tested extensively for their ability to eliminate KRAS localization at cell membranes. Unfortunately, global inhibition of farnesylation comes with numerous off target effects that have prevented broad use of these small molecules. Similarly, specific inhibition of protein kinases with small molecules can be very challenging due to the large size of the human genome and similarities between various kinases. PE offers a potential solution to this specificity problem, as it enables inhibition of modification of the target protein by ablation of the modification site instead of global enzyme inhibition. For example, removal of the lapidated peptide motif in KRAS would be a targeted approach that could be used in place of farnesyl transferase inhibition. This approach is the functional inverse of inhibiting a target protein activity by installing a lipid-targeting motif on a protein not designed to be membrane bound.

[1456] PE can also be used to instigate protein-protein complexation events. Proteins often function within complexes to execute their biological activity. PE can be used to either create or destroy the ability of proteins to exist within these complexes. To eliminate complex formation events, amino acid substitutions or insertions along the protein: protein interface could be installed to disfavor complexation. SSX18 is a protein component of the BAF complex, an important histone-remodeling complex. Mutations in SSX18 drive synovial sarcomas. PE could be used to install side chains that prevent SSX18 from binding to its protein partners in the complex to prevent its oncogenic activity. PE could also be used to remove the pathogenic mutations to restore WT activity of this protein. Alternatively, PE could be used to keep proteins within either their native complex or to drag them to participate in interactions that are unrelated to their native activity to inhibit their activity. Forming complexes that maintain one interaction state over another could represent an

important therapeutic modality. Altering protein: protein interfaces to decrease the Kd of the interaction would keep those proteins stuck to one another longer. As protein complexes can have multiple signaling complexes, like n-myc driving neuroblastoma signaling cascades in disease but otherwise participating in healthy transcriptional control in other cells. PE could be used to install mutations that drive n-myc association with healthy interactions partners and decrease its affinity for oncogenic interaction partners.

References cited in Example 14

[1457] Each of the following references are incorporated herein by reference.

1. Selective Target Protein Degradation via Phthalimide Conjugation. Winter et al. Science. Author manuscript; available in PMC 2016 Jul 8.

[1458] 2. Reversible disruption of mSWI/SNF (BAF) complexes by the SS18-SSX oncogenic fusion in synovial sarcoma. Kadoch and Crabtree. Cell. 2013 Mar 28;153(1):71-85. doi: 10.1016/j.cell.2013.02.036.

3. Ribosomal frameshifting and transcriptional slippage: From genetic steganography and cryptography to adventitious use. Atkins et al. Nucleic Acids Research, Volume 44, Issue 15, 6 September 2016, Pages 7007–7078.

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7. Post-transcriptional gene regulation by mRNA modifications. Zhao et al. Nature Reviews Molecular Cell Biology volume18, pages31–42 (2017).

EXAMPLE 15 - DESIGN AND ENGINEERING OF PEgRNAS

[1459] Described herein is a series of PEgRNA designs and strategies that can improve prime editing (PE) efficiency.

[1460] Prime editing (PE) is a genome editing technology that can replace, insert, or remove defined DNA sequences within a targeted genetic locus using information encoded within a prime editing guide RNA (PEgRNA). Prime editors (PEs) consist of a sequence-programmable DNA binding protein with nuclease activity (Cas9) fused to a reverse transcriptase (RT) enzyme. PEs form complexes with PEgRNAs, which contain the information for targeting specific DNA loci within their spacer sequences, as well as

information specifying the desired edit in an engineered extension built into a standard sgRNA scaffold. PE:PEgRNA complexes bind and nick the programmed target DNA locus, allowing hybridization of the nicked DNA strand to the engineered primer binding sequence (PBS) of the PEgRNA. The reverse transcriptase domain then copies the edit-encoding information within the RT template portion of the PEgRNA, using the nicked genomic DNA as a primer for DNA polymerization. Subsequent DNA repair processes incorporate the newly synthesized edited DNA strand into the genomic locus. While the versatility of prime editing holds great promise as a research tool and potential therapeutic, several limitations in efficiency and scope exist due to the multi-step process required for editing. For example, unfavorable RNA structures that form within the PEgRNA can inhibit the copying of DNA edits from the PEgRNA to the genomic locus. One potential way to improve PE technology is through redesign and engineering of the critical PEgRNA component. Improvements to the design of these PEgRNAs are likely to be necessary for improved PE efficiency, as well as enable installation of longer inserted sequences into the genome.

[1461] Described herein is a series of PEgRNA designs that are envisioned to improve the efficacy of PE. These designs take advantage of a number of previously published approaches for improving sgRNA efficacy and/or stability, as well as utilize a number of novel strategies. These improvements can belong to one or more of a number of different categories:

[1462] (1) Longer PEgRNAs. This category relations to improved designes that enable efficient expression of functional PEgRNAs from non-polymerase III (pol III) promoters, which would enable the expression of longer PEgRNAs without burdensome sequence requirements;

[1463] (2) Core improvements. This category relates to improvements to the core, Cas9-binding PEgRNA scaffold, which could improve efficacy;

[1464] (3) RT processivity. This category relates to modifications to the PEgRNA that improve RT processivity, enabling the insertion of longer sequences at targeted genomic loci; and

[1465] (4) Termini motifs. This category relates to the addition of RNA motifs to the 5′ and/or 3′ termini of the PEgRNA that improve PEgRNA stability, enhance RT processivity, prevent mis-folding of the PEgRNA, or recruit additional factors important for genome editing.

[1466] Described herein are a number of potential such PEgRNA designs in each category. Several of these designs have been previously described for improving sgRNA activity with

Cas9 and are indicated as such. Described herein is also a platform for the evolution of PEgRNAs for given sequence targets that would enable the polishing of the PEgRNA scaffold and enhance PE activity (5). Notably, these designs could also be readily applied to improve PEgRNAs recognized by any Cas9 or evolved variant thereof.

[1467] (1) Longer PEgRNAs.

[1468] sgRNAs are typically expressed from the U6 snRNA promoter. This promoter recruits pol III to express the associated RNA and is useful for expression of short RNAs that are retained within the nucleus. However, pol III is not highly processive and is unable to express RNAs longer than a few hundred nucleotides in length at the levels required for efficient genome editing[183]. Additionally, pol III can stall or terminate at stretches of U's, potentially limiting the sequence diversity that could be inserted using a PEgRNA. Other promoters that recruit polymerase II (such as pCMV) or polymerase I (such as the U1 snRNA promoter) have been examined for their ability to express longer sgRNAs[183]. However, these promoters are typically partially transcribed, which would result in extra sequence 5′ of the spacer in the expressed PEgRNA, which has been shown to result in markedly reduced Cas9:sgRNA activity in a site-dependent manner. Additionally, while pol III-transcribed PEgRNAs can simply terminate in a run of 6-7 U's, PEgRNAs transcribed from pol II or pol I would require a different termination signal. Often such signals also result in polyadenylation, which would result in undesired transport of the PEgRNA from the nucleus. Similarly, RNAs expressed from pol II promoters such as pCMV are typically 5′-capped, also resulting in their nuclear export.

[1469] Previously, Rinn and coworkers screened a variety of expression platforms for the production of long-noncoding RNA- (lncRNA) tagged sgRNAs[183]. These platforms include RNAs expressed from pCMV and that terminate in the ENE element from the MALAT1 ncRNA from humans[184], the PAN ENE element from KSHV[185], or the 3′ box from U1 snRNA[186]. Notably, the MALAT1 ncRNA and PAN ENEs form triple helices protecting the polyA-tail [184, 187]. It is anticipated that, in addition to enabling expression of RNAs, these constructs could also enhance RNA stability (see section iv). Using the promoter from the U1 snRNA to enable expression of these longer sgRNAs[183] was also explored. It is anticipated that these expression systems will also enable the expression of longer PEgRNAs. In addition, a series of methods have been designed for the cleavage of the portion of the pol II promoter that would be transcribed as part of the PEgRNA, adding either a self-cleaving ribozyme such as the hammerhead[188], pistol[189], hatchet[189], hairpin[190], VS[191], twister[192], or

twister sister[192] ribozymes, or other self-cleaving elements to process the transcribed guide, or a hairpin that is recognized by Csy4[193] and also leads to processing of the guide. Also, it is hypothesized that incorporation of multiple ENE motifs could lead to improved PEgRNA expression and stability, as previously demonstrated for the KSHV PAN RNA and element[185]. It is also anticipated that circularizing the PEgRNA in the form of a circular intronic RNA (ciRNA) could also lead to enhanced RNA expression and stability, as well as nuclear localization[194].

[1470] PEgRNA expression platform consisting of pCMV, Csy4 hairping, the PEgRNA, and MALAT1 ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTAGGGTCATGAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTT
CTCAGGTTTTGCTTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGCAAAAGAT
GCTGGTGGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAATCCCTGCGGCGTCT
TTGCTTTGACT (SEQ ID NO: 757)

[1471] PEgRNA expression platform consisting of pCMV, Csy4 hairping, the PEgRNA, and PAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC

ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAA (SEQ ID NO: 758)

[1472] PEgRNA expression platform consisting of pCMV, Csy4 hairping, the PEgRNA, and 3xPAN ENE

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTTGTTTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCA
GACGGCAAGGTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAAT
TTAACGTGCCTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAAC
ATAAATAAAGGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAACACACTGT
TTTGGCTGGGTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAG
GTTTTTATCCCAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGC
CTAGAGCTCAAATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAA
GGTCAATGTTTAATCCATAAAAAAAAAAAAAAAAAAAATCTCTCTGTTTTGGCTGG
GTTTTTCCTTGTTCGCACCGGACACCTCCAGTGACCAGACGGCAAGGTTTTTATCC
CAGTGTATATTGGAAAAACATGTTATACTTTTGACAATTTAACGTGCCTAGAGCTCA
AATTAAACTAATACCATAACGTAATGCAACTTACAACATAAATAAAGGTCAATGTTT
AATCCATAAAAAAAAAAAAAAAAAAA (SEQ ID NO: 759)

[1473] PEgRNA expression platform consisting of pCMV, Csy4 hairping, the PEgRNA, and 3′ box

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTC
CGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC
GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAA
GTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC
CTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTA
CGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCG
TGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATG
GGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGC
AGAGCTGGTTTAGTGAACCGTCAGATCGTTCACTGCCGTATAGGCAGGGCCCAGA
CTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAG
TCTGTTTGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTTGTTTGGTT
TGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 760)

[1474] PEgRNA expression platform consisting of pU1, Csy4 hairping, the PEgRNA, and 3′ box

CTAAGGACCAGCTTCTTTGGGAGAGAACAGACGCAGGGGCGGGAGGGAAAAAG
GGAGAGGCAGACGTCACTTCCCCTTGGCGGCTCTGGCAGCAGATTGGTCGGTTGA
GTGGCAGAAAGGCAGACGGGGACTGGGCAAGGCACTGTCGGTGACATCACGGAC
AGGGCGACTTCTATGTAGATGAGGCAGCGCAGAGGCTGCTGCTTCGCCACTTGCT
GCTTCACCACGAAGGAGTTCCCGTGCCCTGGGAGCGGGTTCAGGACCGCTGATCG
GAAGTGAGAATCCCAGCTGTGTGTCAGGGCTGGAAAGGGCTCGGGAGTGCGCGG
GGCAAGTGACCGTGTGTGTAAAGAGTGAGGCGTATGAGGCTGTGTCGGGGCAGA
GGCCCAAGATCTCAGTTCACTGCCGTATAGGCAGGGCCCAGACTGAGCACGTGAG
TTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGGACCGAGTCGGTCCTCTGCCATCAAAGCGTGCTCAGTCTGTTTCAGCAAG
TTCAGAGAAATCTGAACTTGCTGGATTTTTGGAGCAGGGAGATGGAATAGGAGCT
TGCTCCGTCCACTCCACGCATCGACCTGGTATTGCAGTACCTCCAGGAACGGTGC
ACCCACTTTCTGGAGTTTCAAAAGTAGACTGTACGCTAAGGGTCATATCTTTTTTT
GTTTGGTTTGTGTCTTGGTTGGCGTCTTAAA (SEQ ID NO: 761)

[1475] (2) Core improvements.

[1476] The core, Cas9-binding PEgRNA scaffold can likely be improved to enhance PE activity. Several such approaches have already been demonstrated. For instance, the first pairing element of the scaffold (P1) contains a GTTTT-AAAAC pairing element. Such runs of Ts have been shown to result in pol III pausing and premature termination of the RNA transcript. Rational mutation of one of the T-A pairs to a G-C pair in this portion of P1 has been shown to enhance sgRNA activity, suggesting this approach would also be feasible for PEgRNAs[195]. Additionally, increasing the length of P1 has also been shown to enhance sgRNA folding and lead to improved activity[195], suggesting it as another avenue for the improvement of PEgRNA activity. Finally, it is likely the polishing of the PEgRNA scaffold through directed evolution of PEgRNAs on a given DNA target would also result in improved activity. This is described in section (v).

[1477] PEgRNA containing a 6 nt extension to P1

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGCTCATGAAAATGAGCTAGCAAG
TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTC
TGCCATCAAAGCGTGCTCAGTCTGTTTTTTT (SEQ ID NO: 228)

[1478] PEgRNA containing a T-A to G-C mutation within P1

GGCCCAGACTGAGCACGTGAGTTTGAGAGCTAGAAATAGCAAGTTTAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGTTTTTTT (SEQ ID NO: 229)

[1479] (iii) Improvement of RT processivity via modifications to the template region of the PEgRNA

[1480] As the size of the insertion templated by the PEgRNA increases, it is more likely to be degraded by endonucleases, undergo spontaneous hydrolysis, or fold into secondary

structures unable to be reverse-transcribed by the RT or that disrupt folding of the PEgRNA scaffold and subsequent Cas9-RT binding. Accordingly, it is likely that modification to the template of the PEgRNA might be necessary to affect large insertions, such as the insertion of whole genes. Some strategies to do so include the incorporation of modified nucleotides within a synthetic or semi-synthetic PEgRNA that render the RNA more resistant to degradation or hydrolysis or less likely to adopt inhibitory secondary structures[196]. Such modifications could include 8-aza-7-deazaguanosine, which would reduce RNA secondary structure in G-rich sequences; locked-nucleic acids (LNA) that reduce degradation and enhance certain kinds of RNA secondary structure; 2'-O-methyl, 2'-fluoro, or 2'-O-methoxyethoxy modifications that enhance RNA stability. Such modifications could also be included elsewhere in the PEgRNA to enhance stability and activity. Alternatively or additionally, the template of the PEgRNA could be designed such that it both encodes for a desired protein product and is also more likely to adopt simple secondary structures that are able to be unfolded by the RT. Such simple structures would act as a thermodynamic sink, making it less likely that more complicated structures that would prevent reverse transcription would occur. Finally, one could also imagine splitting the template into two, separate PEgRNAs. In such a design, a PE would be used to initiate transcription and also recruit a separate template RNA to the targeted site via an RNA-binding protein fused to Cas9 or an RNA recognition element on the PEgRNA itself such as the MS2 aptamer. The RT could either directly bind to this separate template RNA, or initiate reverse transcription on the original PEgRNA before swapping to the second template. Such an approach could enable long insertions by both preventing mis-folding of the PEgRNA upon addition of the long template and also by not requiring dissociation of Cas9 from the genome for long insertions to occur, which could possibly be inhibiting PE-based long insertions.

[1481] (iv) Installation of additional RNA motifs at the 5′ or 3′ termini

[1482] PEgRNA designs could also be improved via the installation of additional motifs at either end of the terminus of the RNA. Several such motifs - such as the PAN ENE from KSHV and the ENE from MALAT1 were discussed earlier in part (i)[184,185] as possible means to terminate expression of longer PEgRNAs from non-pol III promoters. These elements form RNA triple helices that engulf the polyA tail, resulting in their being retained within the nucleus[184,187]. However, by forming complex structures at the 3′ terminus of the PEgRNA that occlude the terminal nucleotide, these structures would also likely help prevent exonuclease-mediated degradation of PEgRNAs. Other structural elements inserted at the 3′

terminus could also enhance RNA stability, albeit without enabling termination from non-pol III promoters. Such motifs could include hairpins or RNA quadruplexes that would occlude the 3′ terminus[197], or self-cleaving ribozymes such as HDV that would result in the formation of a 2'-3′-cyclic phosphate at the 3′ terminus and also potentially render the PEgRNA less likely to be degraded by exonucleases[198]. Inducing the PEgRNA to cyclize via incomplete splicing - to form a ciRNA - could also increase PEgRNA stability and result in the PEgRNA being retained within the nucleus[194].

[1483] Additional RNA motifs could also improve RT processivity or enhance PEgRNA activity by enhancing RT binding to the DNA-RNA duplex. Addition of the native sequence bound by the RT in its cognate retroviral genome could enhance RT activity[199]. This could include the native primer binding site (PBS), polypurine tract (PPT), or kissing loops involved in retroviral genome dimerization and initiation of transcription[199]. Addition of dimerization motifs - such as kissing loops or a GNRA tetraloop/tetraloop receptor pair[200] - at the 5′ and 3′ termini of the PEgRNA could also result in effective circularization of the PEgRNA, improving stability. Additionally, it is envisioned that addition of these motifs could enable the physical separation of the PEgRNA spacer and primer, prevention occlusion of the spacer which would hinder PE activity. Short 5′ extensions to the PEgRNA that form a small toehold hairpin in the spacer region could also compete favorably against the annealing region of the PEgRNA binding the spacer. Finally, kissing loops could also be used to recruit other template RNAs to the genomic site and enable swapping of RT activity from one RNA to the other (section iii).

[1484] PEgRNA-HDV fusion

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTCGGTCCTCTGCCATCAAAGC
GTGCTCAGTCTGGGCCGGCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAA
CATGCTTCGGCATGGCGAATGGGACTTTTTTT (SEQ ID NO: 230)

[1485] PEgRNA-MMLV kissing loop

GGTGGGAGACGTCCCACCGGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAA
TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACCGAGTC
GGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACC
TTTTTTT (SEQ ID NO: 231)

[1486] PEgRNA-VS ribozyme kissing loop

GAGCAGCATGGCGTCGCTGCTCACGGCCCAGACTGAGCACGTGAGTTTTAGAGCT
AGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGGACC
GAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTCCATCAGTTGACA
CCCTGAGGTTTTTTT (SEQ ID NO: 232)

[1487] PEgRNA-GNRA tetraloop/tetraloop receptor

GCAGACCTAAGTGGUGACATATGGTCTGGGCCCAGACTGAGCACGTGAGTTTTAG
AGCTAUACGTAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTUACGAAGTGG
GACCGAGTCGGTCCTCTGCCATCAAAGCTTCGACCGTGCTCAGTCTGCATGCGATT
AGAAATAATCGCATGTTTTTTT (SEQ ID NO: 233)

[1488] PEgRNA template switching secondary RNA-HDV fusion

TCTGCCATCAAAGCTGCGACCGTGCTCAGTCTGGTGGGAGACGTCCCACCGGCCG
GCATGGTCCCAGCCTCCTCGCTGGCGCCGGCTGGGCAACATGCTTCGGCATGGCG
AATGGGACTTTTTTT (SEQ ID NO: 234)

[1489] (v) Evolution of PEgRNAs

[1490] It is likely that the PEgRNA scaffold can be further improved via directed evolution, in an analogous fashion to how SpCas9 and base editors have been improved[201]. Directed evolution could enhance PEgRNA recognition by Cas9 or evolved Cas9 variants. Additionally, it is likely that different PEgRNA scaffold sequences would be optimal at different genomic loci, either enhancing PE activity at the site in question, reducing off-target activities, or both. Finally, evolution of PEgRNA scaffolds to which other RNA motifs have been added would almost certainly improve the activity of the fused PEgRNA relative to the unevolved, fusion RNA. For instance, evolution of allosteric ribozymes composed of c-di-GMP-I aptamers and hammerhead ribozymes led to dramatically improved activity[202], suggesting that evolution would improve the activity of hammerhead-PEgRNA fusions as well. In addition, while Cas9 currently does not generally tolerate 5′ extension of the sgRNA, directed evolution will likely generate enabling mutations that mitigate this intolerance, allowing additional RNA motifs to be utilized.

[1491] As described herein, a number of these approaches have already been described for use with Cas9:sgRNA complexes, but no designs for improving PEgRNA activity have been reported. Other strategies for the installation of programmable mutations into the genome include base-editing, homology-directed recombination (HDR), precise microhomology-mediated end-joining (MMEJ), or transposase-mediated editing. However, all of these approaches have significant drawbacks when compared to PEs. Current base editors, while more efficient than existing PEs, can only install certain classes of genomic mutations and can result in additional, undesired nucleotide conversions at the site of interest. HDR is only feasible in a very small minority of cell types and results in comparably high rates of random insertion and deletion mutations (indels). Precise MMEJ can lead to predictable repair of double-strand breaks, but is largely limited to installation of deletions, is very site-dependent, and can also have comparably high rates of undesired indels. Transposase-mediated editing has to date only been shown to function in bacteria. As such improvements to PE represent

possibly the best path forward for the therapeutic correction of a wide-swatch of genomic mutations.

References cited in Example 15

[1492] Each of the following references are cited in Example 15, each of which are incorporated herein by reference.

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EXAMPLE 16 - EXPANDING THE TARGETING SCOPE OF PE USING DNA BINDING PROTEINS OTHER THAN SPCAS9

[1493] Prime editing (PE) using *Streptococcus pyogenes* Cas9 (SpCas9) can efficiently install all single base substitutions, insertions, deletions, and combinations thereof at genomic loci where there is a suitably-placed NGG protospacer adjacent motif (PAM) that SpCas9 can

efficiently bind. The methods described herein broaden the targeting capability of PE by expanding the accessible PAMs and, therefore, the targetable genomic loci accessible for efficient PE. Prime editors using RNA-guided DNA binding proteins other than SpCas9 enable an expanded targettable scope of genomic loci by allowing access to different PAMs. In addition, use of RNA-guided DNA binding proteins smaller than SpCas9 also allows for more efficient viral delivery. PE with Cas proteins or other RNA-guided DNA binding proteins beyond SpCas9 will allow for high efficiency therapeutic edits that were either inaccessible or inefficient using SpCas9-based PE.

[1494] This is expected to be used in situations where SpCas9-based PE is either inefficient due to non-ideal spacing of an edit to relative to an NGG PAM or the overall size of the SpCas9-based construct is prohibitive for cellular expression and/or delivery. Specific disease-relevent loci such as the Huntingtin gene, which has few and poorly located NGG PAMs for SpCas9 near the target region, can easily be targeted using different Cas proteins in the PE system such as SpCas9-VRQR which recognizes an NGA PAM. Smaller Cas proteins will be used to generate smaller PE constructs that can be packaged into AAV vectors more efficiently, enabling better delivery to target tissues. FIG. 61 shows the reduction to practice of prime editing using *Staphylococcus aureus* CRISPR-Cas as the RNA-guided DNA binding protein. NT is untreated control.

[1495] FIGs. 62A-62B provide a demonstration of the importance of the protospacer for efficient installation of a desired edit at a precise location with prime editing. This highlights the importance of alternate PAMs and protospacers as novel features of this technology. "n.d." in FIG. 62A is "not detected."

[1496] FIG. 63 shows the reduction to practice of PE using SpCas9(H840A)-VRQR and SpCas9(H840A)-VRER as the RNA-guided DNA binding protein in a prime editor system. The SpCas9(H840A)-VRQR napDNAbp is disclosed herein as SEQ ID NO: 87. The SpCas9(H840A)-VRER napDNAbp is disclosed herein as SEQ ID NO: 88. The SpCas9(H840A)-VRER-MMLV RT fusion protein is disclosed herein as SEQ ID NO: 516, wherein the MMLV RT comprises the D200N, L603W, T330P, T306K, and W313F substitutions relative to the wild type MMLV RT. The SpCas9(H840A)-VRQR-MMLV RT fusion protein is disclosed herein as SEQ ID NO: 515, wherein the MMLV RT comprises the D200N, L603W, T330P, T306K, and W313F substitutions relative to the wild type MMLV RT. Seven different loci in the human genome are targeted: 4 with the SpCas9(H840A)-

VRQR-MMLV RT prime editor system and 3 with the SpCas9(H840A)-VRER-MMLV RT system. The amino acid sequences of the tested contructs are as follows:

SACAS9-M-MLV RT PRIME EDITOR	MKRTADGSEFESPKKKRKVGKRNYILGLDIGITSVGYGIIDYETR DVIDAGVRLFKEANVENNEGRRSKRGARRLKRRRRHRIQRVKK LLFDYNLLTDHSELSGINPYEARVKGLSQKLSEEEFSAALLHLA KRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYVAELQLE RLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFID TYIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPE ELRSVKYAYNADLYNALNDLNNLVITRDENEKLEYYEKFQIIEN VFKQKKKPTLKQIAKEILVNEEDIKGYRVTSTGKPEFTNLKVYH DIKDITARKEIIENAELLDQIAKILTIYQSSEDIQEELTNLNSELTQE EIEQISNLKGYTGTHNLSLKAINLILDELWHTNDNQIAIFNRLKL VPKKVDLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIIKKYG LPNDIIIELAREKNSKDAQKMINEMQKRNRQTNERIEEIIRTTGK ENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNPFNYEVDHII PRSVSFDNSFNNKVLVKQEEASKKGNRTPFQYLSSSDSKISYETF KKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVD TRYATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFK KERNKGYKHHAEDALIIANADFIFKEWKKLDKAKKVMENQMF EEKQAESMPEIETEQEYKEIFITPHQIKHIKDFKDYKYSHRVDKK PNRELINDTLYSTRKDDKGNTLIVNNLNGLYDKDNDKLKKLIN KSPEKLLMYHHDPQTYQKLKLIMEQYGDEKNPLYKYYEETGN YLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNSRNKVVK LSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYE EAKKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIE VNMIDITYREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNL YEVKSKKHPQIIKKGSGGSSGGSSGSETPGTSESATPESSGGSSG GSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGL AVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGIL VPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVP NPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWR DPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDL ILLQYVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQ ICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF LGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEI KQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRR PVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVI LAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVV ALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHT WYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAEL IALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGLLTSE GKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRM ADQAARKAAITETPDTSTLLIENSSPSGGSKRTADGSEFEPKKKR KV (SEQ ID NO: 660)
SPCAS9(H840A)-VRQR-MALONEY MURINE LEUKEMIA VIRUS	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL

REVERSE TRANSCRIPTASE PRIME EDITOR	AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKQYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 661)
SPCAS9(H840A)-VRER-MALONEY MURINE	MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEY KVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTAR RRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLAL

LEUKEMIA VIRUS REVERSE TRANSCRIPTASE PRIME EDITOR	AHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL GLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADL FLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTL LKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPI LEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAIL RRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTR KSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKH SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKT NRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLL KIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFD DKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDG FANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSP AIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQ KNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQN GRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSD KNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKA ERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDEND KLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNA VVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRD FATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARK KDWDPKKYGGFVSPTVAYSVLVVAKVEKGKSKKLKSVKELLGI TIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRK RMLASARELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHR DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKEYRSTKEVL DATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESAT PESSGGSSGGSSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQA WAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKP HIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVN KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPT SQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFNEALHRDL ADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALLQTLGNLG YRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPT PKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWG PDQQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDT DRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTD QPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPA GTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHS AEARGNRMADQAARKAAITETPDTSTLLIENSSPSGGSKRTADG SEFEPKKKRKV (SEQ ID NO: 662)

[1497] As shown in FIG. 63, the SpCas9(H840A)-VRQR-MMLV RT was operational at PAM sites that included "AGAG" and "GGAG", with some editing activity at "GGAT" and

"AGAT" PAM sequences. The SpCas9(H840A)-VRER-MMLV RT was operational at PAM sites that included "AGCG" and "GGCG", with some editing activity at "TGCG."

[1498] The data demonstrates that prime editing may be conducted using napDNAbps which bear different PAM specificities, such as those Cas9 variant described herein.

EXAMPLE 17 – INTRODUCTION OF RECOMBINASE TARGET SITES WITH PE

[1499] This Example describes a method to address genetic disease or generate tailor-made animal or plant models by using prime editing (PE) to introduce recombinase targets sites (SSR target sites) in mammalian and other genomes with high specificity and efficiency.

[1500] This Example describes use of PE to introduce recombinase recognition sequences at high-value loci in human or other genomes, which, after exposure to site-specific recombinase(s), will direct precise and efficient genomic modifications (FIG. 64). In various embodiments show in FIG. 64, PE may be used to (b) insert a single SSR target for use as a site for genomic integration of a DNA donor template. (c) shows how a tandem insertion of SSR target sites can be used to delete a portion of the genome. (d) shows how a tandem insertion of SSR target sites can be used to invert a portion of the genome. (e) shows how the insertion of two SSR target sites at two distal chromosomal regions can result in chromosomal translocation. (f) shows how the insertion of two different SSR target sites in the genome can be used to exchange a cassette from a DNA donor template. Each of the types of genome modifications are envisioned by using PE to insert SSR tagets, but this list also is not meant to be limiting.

[1501] Many large-scale genomic changes, such as gene insertions, deletions, inversions, or chromosomal translocations, are implicated in genetic disease[1-7]. In addition, custom and targeted manipulation of eukaryotic genomes is important for research into human disease, as well as generation of transgenic plants[8,9] or other biotechnological products. For example, microdeletions of chromosomes can lead to disease, and replacement of these deletions by insertions of critical DNA elements could lead to a permanent amelioration of disease. In addition, diseases resulting from inversions, gene copy number changes, or chromosomal translocations could be addressed by restoring the previous gene structure in affected cells. Alternatively, in plants or other high value eukaryotic organisms used in industry, introduction of recombinant DNA or targeted genomic rearrangements could lead to improved products, for example crops which require fewer resources or are resistant to pathogens. Current technologies for effecting large-scale genomic changes rely on random or

stochastic processes, for example the use of transposons or retroviruses, while other desired genomic modifications have only been achieved by homologous recombination strategies.

[1502] One appealing class of proteins for accomplishing targeted and efficient genomic modification is site-specific recombinases (SSRs). SSRs have a long history of being used as a tool for genomic modification[10-13]. SSRs are considered promising tools for gene therapy because they catalyze the precise cleavage, strand exchange, and rejoining of DNA fragments at defined recombination targets[14] without relying on the endogenous repair of double-strand breaks which can induce indels, translocations, other DNA rearrangements, or p53 activation[15-18]. The reactions catalyzed by SSRs can result in the direct replacement, insertion, or deletion of target DNA fragments with efficiencies exceeding those of homology-directed repair[14,19].

[1503] Although SSRs offer many advantages, they are not widely used because they have a strong innate preference for their cognate target sequence. The recognition sequences of SSRs are typically $\geq$ 20 base pairs and thus unlikely to occur in the genomes of humans or model organisms. Further, the native substrate preferences of SSRs are not easily altered, even with extensive laboratory engineering or evolution[20]. This limitation is overcome by using PE to directly introduce recombinase targets into the genome, or to modify endogenous genomic sequences which natively resemble recombinase targets. Subsequent exposure of the cell to recombinase protein will permit precise and efficient genomic modification directed by the location and orientation of the recombinase target(s) (FIG. 64).

[1504] PE-mediated introduction of recombinase targets could be particularly useful for the treatment of genetic diseases which are caused by large-scale genomic defects, such as gene loss, inversion, or duplication, or chromosomal translocation[1-7] (Table 6). For example, Williams-Beuren syndrome is a developmental disorder caused by a deletion of 24 in chromosome 721. No technology exists currently for the efficient and targeted insertion of multiple entire genes in living cells (the potential of PE to do such a full-length gene insertion is currently being explored but has not yet been established); however, recombinase-mediated integration at a target inserted by PE offers one approach towards a permanent cure for this and other diseases. In addition, targeted introduction of recombinase recognition sequences could be highly enabling for applications including generation of transgenic plants, animal research models, bioproduction cell lines, or other custom eukaryotic cell lines. For example, recombinase-mediated genomic rearrangement in transgenic plants at PE-specific targets

could overcome one of the bottlenecks to generating agricultural crops with improved properties8,9.

[1505] A number of SSR family members have been characterized and their target sequences described, including natural and engineered tyrosine recombinases (TABLE 7), large serine integrases (TABLE 8), serine resolvases (TABLE 9), and tyrosine integrases (TABLE 10). Modified target sequences that demonstrate enhanced rates of genomic integration have also been described for several SSRs[22-30]. In addition to natural recombinases, programmable recombinases with distinct specificities have been developed[31-40]. Using PE, one or more of these recognition sequences could be introduced into the genomic at a specified location, such as a safe harbor locus[41-43], depending on the desired application.

[1506] For example, introduction of a single recombinase target in the genome would result in integrative recombination with a DNA donor template (FIG. 64b). Serine integrases, which operate robustly in human cells, may be especially well-suited for gene integration[44,45]. Additionally, introduction of two recombinase targets could result in deletion of the intervening sequence, inversion of the intervening sequence, chromosomal translocation, or cassette exchange, depending on the identity and orientation of the targets (FIG. 64c-f). By choosing endogenous sequences that already closely resemble recombinase targets, the scope of editing required to introduce the complete recombinase target would be reduced.

[1507] Finally, several recombinases have been demonstrated to integrate into human or eukaryotic genomes at natively occurring pseudosites[46-64]. PE editing could be used to modify these loci to enhance rates of integration at these natural pseudosites, or alternatively, to eliminate pseudosites that may serve as unwanted off-target sequences.

[1508] This report describes a general methodology for introducing recombinase target sequences in eukaryotic genomes using PE, the applications of which are nearly limitless. The genome editing reactions are intended for use with "prime editor," a chimeric fusion of a CRISPR/Cas9 protein and a reverse-transcriptase domain, which utilizes a custom prime editing guide RNA (PEgRNA). By extension, Cas9 tools and homology-directed repair (HDR) pathways may also be exploited to introduce recombinase targets through DNA templates by lowering the rates of indels using several techniques[65-67]. A proof-of-concept experiment in human cell culture is shown in FIG. 65.

Table 6. Examples of genetic diseases linked to large-scale genomic modifications.

Disease	Source	Cause

Trisomy 17p	68	Gene duplication
Charcot-Marie-Tooth	69	Gene duplication
Smith-Magenis syndrome	70	Gene deletion
Williams-Beuren	21	Gene deletion
De la Chapelle syndrome	71	Chromosomal translocation
Down syndrome (some	72	Chromosomal translocation
Hemophilia A	73	Gene inversion
Hunter syndrome	74	Gene inversion

Table 7. Tyrosine recombinases and SSR target sequences.

cRecombinase	**Source**	**Target**	**Name**
Cre	75	ATAACTTCGTATAGCATACATTATACG AAGTTAT (SEQ ID NO: 517)	*loxP*
Dre	76	TAACTTTAAATAATGCCAATTATTTAA AGTTA (SEQ ID NO: 518)	*rox*
VCre	77	TCAATTTCTGAGAACTGTCATTCTCGG AAATTGA (SEQ ID NO: 519)	*loxV*
SCre	77	CTCGTGTCCGATAACTGTAATTATCGG ACATGAT (SEQ ID NO: 520)	*loxS*
Flp	78	GAAGTTCCTATTCTCTAGAAAGTATAG GAACTTC (SEQ ID NO: 521)	*FRT*
B2	79	GAGTTTCATTAAGGAATAACTAATTCC CTAATGAAACTC (SEQ ID NO: 522)	*loxB*
B3	79	GGTTGCTTAAGAATAAGTAATTCTTAA GCAACC (SEQ ID NO: 523)	*loxB3*
Kw	80	ACGAAAAATGGTAAGGAATAGACCAT TCCTTACCATTTTTGGT (SEQ ID NO: 524)	
R	81	TTGATGAAAGAATAACGTATTCTTTCA TCAA (SEQ ID NO: 525)	*RS*
TD1-40	82	GTGCGTCAAATAATAACGTATTATTTG ACACTT (SEQ ID NO: 526)	*TDRS*
Vika	83	AATAGGTCTGAGAACGCCCATTCTCAG ACGTATT (SEQ ID NO: 527)	*vox*
Nigri	84	TGAATGTCCTATAATTACACTTATAGG ACATTCA (SEQ ID NO: 528)	*nox*
Panto	84	GAAACTTTAAATAATAAGTCTTATTTA AAGTTTC (SEQ ID NO: 259)	*pox*

Kd	79	AAACGATATCAGACATTTGTCTGATA ATGCTTCATTATCAGACAAATGTCTG ATATCGTTT (SEQ ID NO: 530)	*loxK*
Fre	85	ATATATACGTATATAGACATATATACG TATATAT (SEQ ID NO: 531)	*loxH*
CreALSHG	86	ATAACTCTATATAATGTATGCTATATA GAGTTAT (SEQ ID NO: 532)	*loxM7*
Tre	87	ACAACATCCTATTACACCCTATATGCC AACATGG (SEQ ID NO: 533)	*loxLTR*
Brec1	12	AACCCACTGCTTAAGCCTCAATAAAGC TTGCCTT (SEQ ID NO: 534)	*loxBTR*
Cre-R3M3	88	GATACAACGTATATACCTTTCTATACG TTGTTTA (SEQ ID NO: 535)	*loxK2*

Table 8. Large serine integrases and SSR target sequences.

Integrase	Source	Left Target	Right Target
Bxb1	89	GGTTTGTCTGGTCAACCACC GCGGTCTCAGTGGTGTACGG TACAAACC (SEQ ID NO: 536)	GGCTTGTCGACGACGGCGG TCTCCGTCGTCAGGATCAT(S EQ ID NO: 537)
phiC31	90	GTGCCCCAACTGGGGTAACC TTTGAGTTCTCTCAGTTGGG GG (SEQ ID NO: 538)	TGCGGGTGCCAGGGCGTGC CCTTGGGCTCCCCGGGCGCG TACTCC (SEQ ID NO: 539)
R4	91	TGTTCCCCAAAGCGATACCA CTTGAAGCAGTGGTACTGCT TGTGGGTACA (SEQ ID NO: 540)	GCATGTTCCCCAAAGCGATA CCACTTGAAGCAGTGGTACT GCTTGTGGGTACACTCTGCG GGTG (SEQ ID NO: 541)
phiBT1	92	GGTGCTGGGTTGTTGTCTCT GGACAGTGATCCATGGGAA ACTACTCAGCACC (SEQ ID NO: 542)	CAGGTTTTTGACGAAAGTGA TCCAGATGATCCAG (SEQ ID NO: 543)
MJ1 (phiFC1)	93	ATTTTAGGTATATGATTTTGT TTATTAGTGTAAATAACACT ATGTACCTAAAAT (SEQ ID NO: 544)	CAAAGGATCACTGAATCAA AAGTATTGCTCATCCACGCG AAA (SEQ ID NO: 545)
MR11	94	TTTGTGCGGAACTACGAACA GTTCATTAATACGAAGTGTA CAAACTTCCATACAA (SEQ ID NO: 546)	CGAAAATGTATGGAGGCAC TTGTATCAATATAGGATGTA TACCTTCGAAGACACTT (SEQ ID NO: 547)
TP901-1	95	GAGTTTTTATTTCGTTTATTT CAATTAAGGTAACTAAAAA ACTCCTTTTAAGG (SEQ ID NO: 548)	ATGCCAACACAATTAACATC TCAATCAAGGTAAATGCTTT TTGCTTTTTTTGC (SEQ ID NO: 549)

A118	96	TTCCTCGTTTTCTCTCGTTGGAAGAAGAAGAAACGAGAAA (SEQ ID NO: 550)	TTTCGGATCAAGCTATGAAGGACGCAAAGAGGGAACTAAA(SEQ ID NO: 551)
U153	97	TTCCTCGTTTTCTCTCGTTGGACGGAAACGAATCGAGAAA (SEQ ID NO: 552)	TTTCGGATCAAGCTATGAAGGACGCAAAGAGGGAACTAAA (SEQ ID NO: 553)
phiRV1	98	GTAGTGTATCTCACAGGTCCACGGTTGGCCGTGGACTGCTGAAGAACATTCC (SEQ ID NO: 554)	GAAGGTGTTGGTGCGGGGTTGGCCGTGGTCGAGGTGGGGT (SEQ ID NO: 555)
phi370.1	99	AAAAAAATACAGCGTTTTTCATGTACAACTATACTAGTTGTAGTGCCTAAAA (SEQ ID NO: 556)	TTGTAAAGGAGACTGATAATGGCATGTACAACTATACTCGTCGGTAAAAAGGCA (SEQ ID NO: 557)
TG1	100	TCCAGCCCAACAGTGTTAGTCTTTGCTCTTACCCAGTTGGGCGGGA (SEQ ID NO: 558)	GATCAGCTCCGCGGGCAAGACCTTTCTCCTTCACGGGGTGGAAGGTC (SEQ ID NO: 559)
WB	101	CTAGTTTTAAAGTTGGTTATTAGTTACTGTGATATTTATCACGGTACCCAATAACCAATGAAT (SEQ ID NO: 560)	CGGAAGGTAGCGTCAACGATAGGTGTAACTGTCGTGTTTGTAACGGTACTTCCAACAGCTGGCGCCGCCAC (SEQ ID NO: 561)
BL3	102	CAATGAAAAACTAGGCATGTAGAAGTTGTTTGT (SEQ ID NO: 562)	TTTCCACAGACAACTCACGTGGAGGTAGTCAC (SEQ ID NO: 563)
SprA	103	TGTAGTAAGTATCTTAATATACAGCTTTATCTGTTTTTTAAGATACTTACTACTTT (SEQ ID NO: 564)	CACCCATTGTGTTCACAGGAGATACAGCTTTATCTGTACTGATATTAATGACATGCTG (SEQ ID NO: 565)
phiJoe	104	AGTTGTGGCCATGTGTCCATCTGGGGGCAGATGGAGACGGGGTCACA (SEQ ID NO: 566)	ATCTGGATGTGGGTGTCCATCTGCGGGCAGACGCCGCAGTCGAAGCACGG (SEQ ID NO: 567)
phiK38	105	CCCTAATACGCAAGTCGATAACTCTCCTGGGAGCGTTGACAACTTGCGCACCCTGATCTG (SEQ ID NO: 569)	GAGCGCCGGATCAGGGAGTGGACGGCCTGGGAGCGCTACACGCTGTGGCTGCGGTCGGTGC (SEQ ID NO: 570)
Int2	105	GCTCATGTATGTGTCTACGCGAGATTCTCGCCCGAGAACTTCTGCAAGGCACTGCTCTTGGCT (SEQ ID NO: 571)	GGACGGCGCAGAAGGGGAGTAGCTCTTCGCCGGACCGTCGACATACTGCTCAGCTCGTC (SEQ ID NO: 572)
Int3	105	ATGGATAAAAAAATACAGCGTTTTTCATGTACAACTATACTAGTTGTAGTGCCTAAATA	GTTTGTAAAGGAGACTGATAATGGCATGTACAACTATACTCGTCGGTAAAAAGGCATCT

		ATGCTT (SEQ ID NO: 573)	TAT (SEQ ID NO: 574)
Int4	105	AAAAATTACAAAGTTTTCAACCCTTGATTTGAATTAGCGGTCAAATAATTTGTAATTCGTTT (SEQ ID NO: 575)	TTCCAAAGAGCGCCCAACGCGACCTGAAATTTGAATAAGACTGCTGCTTGTGTAAAGGCGATGATT (SEQ ID NO: 576)
Int7	105	GTGTTATAAACCTGTGTGAGAGTTAAGTTTACATGCCTAACCTTAACTTTTACGCAGGTTCAGCTT(SEQ ID NO: 577)	AGACGAGAAACGTTCCGTCCGTCTGGGTCAGTTGGGCAAAGTTGATGACCGGGTCGTCCGTT (SEQ ID NO: 578)
Int8	105	TTAATAAACTATGGAAGTATGTACAGTCTTGCAATGTTGAGTGAACAAACTTCCATAATAAAAT (SEQ ID NO: 579)	CAATCATCAGATAACTATGGCGGCACGTGCATTAACCACGGTTGTATCCCGTCTAAAGTACTCGT (SEQ ID NO: 580)
Int9	105	GTGGTTGTTTTTGTTGGAAGTGTGTATCAGGTATCTGCATAGTTATTCCGAACTTCCAATTA (SEQ ID NO: 581)	TTTATATTGCGAAAAATAATTGGCGAACGAGGTAACTGGATACCTCATCCGCCAATTAAAATTTG (SEQ ID NO: 582)
Int10	105	GGAAAATATAAATAATTTTAGTAACCTACATCTCAATCAAGGATAGTAAAACTCTCACTCTT (SEQ ID NO: 583)	AGCACGCTGATAATCAGCAAGACCACCAACATTTCCACCAATGTAAAAGCTTTAACCTTAGC (SEQ ID NO: 584)
Int11	105	GTTTATATGTTTACTAATAAGACGCTCTCAACCCATAAAGTCTTATTAGTAAACATATTTCAACT (SEQ ID NO: 585)	ATGGATTTTGCAGATTCCCAGATGCCCCTACAGAAAGAGGTACAAAACATTTATTGGAATTAATT (SEQ ID NO: 586)
Int12	105	TTTTTGTATGTTAGTTGTGTCACTGGGTAGACCTAAATAGTGACACAACTGCTATTAAAATTTAA (SEQ ID NO: 587)	GTTCGTGGTAACTATGGGTGGTACAGGTGCCACATTAGTTGTACCATTTATGTTTATGTGGTTAAC (SEQ ID NO: 588)
Int13	105	CAATAACGGTTGTATTTGTAGAACTTGACCAGTTGTTTTAGTAACATAAATACAACTCCGAATA (SEQ ID NO: 589)	GCATACATTGTTGTTGTTTTTCCAGATCCAGTTGGTCCTGTAAATATAAGCAATCCATGTGAGT (SEQ ID NO: 590)
LI	106	GTTTAGTATCTCGTTATCTCTCGTTGGAGGGAGAAGAAACGGGATACCAAAA (SEQ ID NO: 591)	TAACTTTTTCGGATCGAGTTATGATGGACGTAAAGAGGGAACAAAGCATCTA (SEQ ID NO: 592)
Peaches	107	TAGTTTCCAATGTTACAGGAACTGCTGGCAGAATCCAACACATTGGAAGTCG (SEQ ID NO: 593)	CGGTCTCCATCGGGATCTGCTGATCGAGCAGCATGCCGACCA (SEQ ID NO: 594)
Bxz2	107	TAACCGCAAGTGTACATCCCTCGGCTGGCCGAGACAAGTACAGTTGCGACAG (SEQ ID	CGGTCTCCATCGGGATCTGCTGATCGAGCAGCATGCCGACCA (SEQ ID NO: 596)

		NO: 595)	
SV1	108	ATGTGGTCCTTTAGATCCAC TGACGTGGGTCAGTGTCTCT AAAGGACTCGCG (SEQ ID NO: 597)	CATCAGGGCGGTCAGGCCG TAGATGTGGAAGAAACGGC AGCACGGCGAGGACG (SEQ ID NO: 598)

Table 9. Serine resolvases and SSR target sequences.

Resolvase	Source	Left Target	Right Target
Gin	109	CGTTTCCTGTAAACCGAGGT TTTGGATAAACA (SEQ ID NO: 599)	CGTTTCCTGTAAACCGAGGT TTTGGATAATGG (SEQ ID NO: 600)
Cin	110	GAGTTCTCTTAAACCAAGGT TTAGGATTGAAA (SEQ ID NO: 601)	GAGTTCTCTTAAACCAAGGT ATTGGATAACAG (SEQ ID NO: 602)
Hin	111	TGGTTCTTGAAAACCAAGGT TTTTGATAAAGC (SEQ ID NO: 603)	AAATTTTCCTTTTTGGAAGG TTTTTGATAACCA (SEQ ID NO: 604)
Min	112	GCCTTCCCCTAAACCAACGT TTTTATGCCGCC (SEQ ID NO: 605)	GCCTTCCCCCAAACCAAGGT AATCAAGAACGC (SEQ ID NO: 606)
Sin	113	TTGTGAAATTTGGGTACACC CTAATCATACAA (SEQ ID NO: 607)	CGTATGATTAGGGTGTATAT TAATTT (SEQ ID NO: 608)

Table 10. Tyrosine integrases and target sequences.

Integrase	Source	*attP*	*attB*
HK022	114	CAAATGATTTTATTTTGACTAATAA TGACCTACTTACATTAATTTACTGAT AATTAAAGAGATTTTAAATATACAA CTTATTCACCTAAAGGATGACAAAA (SEQ ID NO: 609)	GCACTTTAGGTGA AAAAGGTT (SEQ ID NO: 610)
P22	115	CTAAGTGGTTTGGGACAAAAATGGG ACATACAAATCTTTGCATCGGTTTG CAAGGCTTTGCATGTCTTTCGAAGA TGGGACGTGTGAGCGCAGGTATGAC GTGGTATGTGTTGACTTAAAAGGTA GTTCTTATAATTCGTAATGCGAAGG TCGTAGGTTCGACTCCTATTATCGG CACCAGTTAAATCAAATACTTACGT ATTATTCGTGCCTTCCTTATTTTTAC TGTGGGACATATTTGGGACAGAAGT ACCAAAAA (SEQ ID NO: 612)	GCAGCGCATTCGT AATGCGAAGGTCG T (SEQ ID NO: 613)
L5	116	GCGATCCCCATCCGCGACGTGCCAA	GAGCGGGCGACG

		CTAGGTCTCCTCTCGTCGTGAACAA GGCTACCGGGTTGCAACTCCTGTGC AACTCTCAGGCTTCAACGCGCTTCT ACGACCTGCAATTTCTTTCCACTTA GAGGATGCAGCCGAGAGGGGTAAA AACCTATCTTGACCGGCCCATATGT GGTCGGCAGACACCCATTCTTCCAA ACTAGCTACGCGGGTTCGATTCCCG TCGCCCGCTCCGCTGGTCAGAGGGT GTTTTCGCCCTCTGGCCATTTTTCTT TCCAGGGGTCTGCAACTCTTGTGCG ACTCTTCTGACCTGGGCATACGCGG TTGCAACGCATCCCTGATCTGGCTA CTTTCGATGCTGACAAACGAATAGA GCCCCCCGCCTGCGCGAACAGACG AGGGGCATTCACA (SEQ ID NO: 614)	GGAATCGAACCCG CGTAGCTAGTTTG GAAGA (SEQ ID NO: 615)

References cited in Example 17

[1509] Each of the following references are cited in Example 17, each of which are incorporated herein by reference.

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EXAMPLE 18 – INCORPORATION OF 3′ TOELOOP IN THE PRIMER BINDING SITE (PBS) IMPROVES PEgRNA ACTIVITY

[1510] In order to further improve PE activity, the inventors contemplated adding a toeloop sequence at the 3′ end of a PEgRNA having a 3′ extension arm. FIG. 71A provides an example of a generic SpCas9 PEgRNA having a 3′ extension arm (top molecule). The 3′ extension arm, in turn, comprises an RT template (that includes that the desired edit) and a primer binding site (PBS) at the 3′ end of the molecule. The molecule terminates with a poly(U) sequence comprising three U nucleobases (i.e., 5′-UUU-3′).

[1511] By contrast, the bottom portion of FIG. 71A shows the same PEgRNA molecule as the top portion of FIG. 71A, but wherein a 9-nucleobase sequence of 5′-GAAANNNNN-3′

has been inserted between the 3′ end of the primer binding site and the 5′ end of the terminal poly(U) sequence. This structure folds back on itself by 180° to form a "toeloop" RNA structure, wherein the sequences of 5′-NNNNN-3′ of the 9-nucleobase insertion anneals with a complementary sequence in the primer binding site, and wherein the 5′-GAAA-3′ portion forms the 180° turn. The features of the toeloop sequence depicted in FIG. 71A is not intended to limit or narrow the scope of possible toeloops that could be used in its place. Further, the sequence of the toeloop will depend upon the complementary sequence of the primer binding site. Essentially though, the toeloop sequence, in various embodiments, may have a first sequence portion that forms a 180°, and a second sequence portion that has a sequence that is complentary to a portion of the primer binding site.

[1512] Without being bound by theory, the toeloop sequence is thought to enable PEgRNAthe use of PEgRNAs with increasingly longer primer binding sites than would otherwise be possible. Longer PBS sequences, in turn, are thought to improve PE activity. PEgRNA More in particular, the likely function of the toeloop is to occlude or at least minimize the PBS from interacting with the spacer. Stable hairpin formation between the PBS and the spacer can lead to an inactive PEgRNA. Without a toeloop, this interaction may require restricting the length of the PBS. Blocking or minimizing the interaction between the spacer and the PBS using a 3' end toeloop may lead to an improvement in PE activity.

EXAMPLE 19 – PRIME EDITING WITH ALTERNATIVE NUCLEIC ACID TEMPLATES AND EDITOR PROTEIN CONSTRUCTS

[1513] Prior to this example, prime editing is described as requiring a PEgRNA. Exemplary embodiments describing possible configurations of suitable PEgRNA for use in prime editing are depicted in FIG. 3A (a PEgRNA with a 5' extension arm), FIG. 3B (a PEgRNA with a 3' extension arm), FIG. 3C (an internally extended PEgRNA), FIG. 3D (a PEgRNA with a 3' extension arm, and comprising a primer binding site, edit template, homology arm, and optional 3' and 5' modifier regions, and a region indicated as the DNA synthesis template), and FIG. 3E (a PEgRNA with a 5' extension arm, and comprising a primer binding site, edit template, homology arm, and optional 3' and 5' modifier regions, and a region indicated as the DNA synthesis template). In addition, PEgRNA structure and composition are described extensively herein in the Detailed Description and throughout.

[1514] This Example describes additional design variations of PEgRNAs—in some cases, PEgRNAs which are wholly or partially chemically synthesized outside the cell—that are

envisioned to work in conjunction with the prime editors of this Specification. Such alternative designs may improve various aspects of prime editing, including the insertion of longer DNA sequences by prime editing, the use of alternative polymerases (i.e., alternatives to reverse transcriptase) that potentially operate with increased efficiency and/or fidelity, and the use or recruitment of alternative and/or addition prime editor protein effector components to enhance or augment prime editing. In addition, the use of chemically synthesized PEgRNAsPEgRNA may potentially lead to the production of molecules that are more stable and possess desirable features that enhance prime editing efficiency and capabilities.

[1515] PEgRNAPEgRNAPEgRNAA PEgRNA serves as the nucleic acid template that encodes the desired edited genetic information that is to be incorporated into a target site. In one aspect, a PEgRNA is created by adding an extension arm to either the 5' end or 3' end of an sgRNA (e.g., as shown in the embodiments of FIG. 3A, 3B, 3D, or 3E), or by inserting a similar sequence internally within an sgRNA (e.g., as shown in the embodiment of FIG. 3C), wherein the extension arm comprises a DNA synthesis template that is capable of encoding a ssDNA product by a polymerase (e.g., a reverse transcriptase) and which includes the edited genetic information of interest. The extension arm comprises a primer binding site (PBS) for annealing to the napDNAbp-nicked genomic DNA strand, and a DNA synthesis template to encode t the edited DNA strand of interest, which becomes incorporated into the endogenous DNA target site by replacing the counterpart DNA strand. PEgRNA can be expressed within cells from plasmid DNA or a genomically integrated DNA cassette, or they can be made outside of cells by *in vitro* transcription or by chemical synthesis and subsequently delivered into cells. Preparation of PEgRNAs outside of cells, particularly by chemical synthesis, offers an opportunity to modify the PEgRNAs substantially. This invention describes alternative designs for prime editing templates (Fig. 72).

(A) DNA synthesis template expressed as a separate molecules from guide RNAs (i.e., DNA synthesis template provided *in trans* to the prime editor complex (napDNAbp + guide RNA).

[1516] In various embodiments described herein, prime editing utilizes a single PEgRNA that serves as both the programmable targeting molecules and the edit-encoding molecule. This embodiment is depicted in FIG. 72(a) with a PEgRNA having a 3' extension arm. However, in some cases, this could be disadvantageous, particularly for more complex PEgRNA molecules such as those that encode a large insertion. These RNAs could contain extensive secondary structure that interferes with the PEgRNA scaffold structure and interactions with Cas9. Alternatively, prime editing can be carried out by substituting a PEgRNA with two

separate RNA molecules: an sgRNA, and a *trans* prime editing RNA template (tPERT), as depicted in FIG. 72(b). The sgRNA serves to target Cas9 (or more generally, the napDNAbp) to the desired genomic target site, while the tPERT is used by the polymerase (e.g., a reverse transcriptase) to write new DNA sequence into the target locus.

[1517] In general, simple expression of a tPERT leads to lower editing efficiency compared to a PEgRNA. However, the efficiency of *trans* prime editing can be enhanced by the introduction of one or more MS2 RNA aptamers into the tPERT RNA, along with a fusion of the MS2 coat protein (MS2cp) to the prime editor protein (to make MS2cp-Cas9-RT). This allows for the MS2 RNA aptamer to bind to the MS2cp, thereby co-localizing the tPERT (which comprises the DNA synthesis template) to the site of editing by the prime editor complex. The MS2 aptamer is preferably placed on the 3' end of the tPERT to avoid reverse transcription of the aptamer sequence.

[1518] Although this example utilizes the MS2 tagging technique (comprising the MS2 RNA aptamer on the tPERT paired with the MS2cp protein fused to the prime editor), other RNA-protein recruitment systems can be used in the alternative. The general concept envisioned here is that the DNA synthesis template of the tPERT is modified to contain an RNA recruitment secondary structure (e.g., a specialized hairpin like the MS2 aptamer) so that the tPERT may be recruited by a modified prime editor fusion protein that further comprises an RNA-binding protein that specifically recognizes and binds to the RNA recruitment second structure on the tPERT molecule. A review of other RNA-protein recruitment domains are described in the art, for example, in Johansson et al., "RNA recognition by the MS2 phage coat protein," *Sem Virol.*, 1997, Vol. 8(3): 176-185; Delebecque et al., "Organization of intracellular reactions with rationally designed RNA assemblies," *Science*, 2011, Vol. 333: 470-474; Mali et al., "Cas9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering," *Nat. Biotechnol.*, 2013, Vol.31: 833-838; and Zalatan et al., "Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds," *Cell*, 2015, Vol.160: 339-350, each of which are incorporated herein by reference in their entireties. Other systems include the PP7 hairpin, which specifically recruits the PCP protein, and the "com" hairpin, which specifically recruits the Com protein. See Zalatan et al. Any of these well-known recruitment systems may be employed with *trans* prime editing as described herein.

[1519] The efficiency of the instant tPERT *trans* prime editing system was tested. Up to 20% efficiency of His6 insertion (18 bp) was achieved at the HEK3 site in HEK293T cells

using a tPERT containing a single 3' MS2 aptamer, a 13-nt primer binding site, and an RT template containing the insertion sequence and 34 nt of homology to the locus, along with an editor containing the MS2cp fused to the N-terminus of PE2. See FIG. 73. The strategy of *trans* prime editing has the potential to address complications associated with PEgRNAPEgRNA design, and could be more suited for longer RT templates to achieve larger insertion, deletions, or edits at further distances from the prime editor nick site.

(B) Chemically synthesized PEgRNAs with RNA and DNA synthesis templates

[1520] Alternative nucleic acid templates can be used within chemically synthesized PEgRNAs (FIG. 72c). For example, a synthetic PEgRNA can be constructed as an RNA/DNA hybrid wherein the spacer sequence and sgRNA scaffold is composed of RNA nucleotides and the primer binding site and synthesis template (shown as a 3' extension in FIG. 72c) is composed of DNA nucleotides. This could allow for DNA-dependent DNA polymerases to be used in place of reverse transcriptase within prime editors. It could also prevent the synthesis of DNA that is templated by sgRNA scaffold sequence. In other designs, chemical linkers, composed of non-templating nucleotides or other suitable linker moieties, can be used to tether the nucleic acid edit template (composed of RNA or DNA) to the sgRNA scaffold. This could prevent continued DNA polymerization of the sgRNA scaffold and allow for flexibility in the extension that allows for more efficient templated synthesis. Finally, the directionality of the nucleic acid synthesis template can be inverted such that DNA polymerization proceeds away from the sgRNA scaffold as opposed to toward it.

(C) Recruitment of the DNA polymerase expressed i*n trans*

[1521] In the main embodiment of prime editing, the polymerase (e.g., reverse transcriptase enzyme) is expressed as a fusion to the napDNAbp (e.g., Cas9 nickase). Alternatively, the polymerase (e.g., reverse transcriptase) can be expressed *in trans*, and its activity can be localized to the editing site using recruitment systems such as the MS2 RNA aptamer and MS2 coat protein, or other similar recruitment system known in the art. In this system, the PEgRNA is modified to include an MS2 aptamer within one of the sgRNA scaffold hairpins, and the polymerase (e.g., reverse transcriptase) is expressed as a fusion protein to MS2cp. The napDNAbp (e.g., Cas9 nickase) is also expressed as an independent polypeptide. This system has been demonstrated with the wild type M-MLV reverse transcriptase (FIG. 74), and should be applicable to other RT variants. In addition, other RNA-protein interactions, or protein-protein interactions, could be used for RT recruitment.

The following sequences are pertinent to Example 19:

Sequences of tPERTs:

MS2 aptamer / **RT template** / PBS / **Linker**

5'-MS2_13nt-PBS:

5'GCCAACATGAGGATCACCCATGTCTGCAGGGCC**TGGAGGAAGCAGGGCTTCC TTTCCTCTGCCATCAATGATGGTGATGATGGTG**CGTGCTCAGTCTG – 3' (SEQ ID NO: 762)

5'-MS2_17nt-PBS:

5'GCCAACATGAGGATCACCCATGTCTGCAGGGCC**TGGAGGAAGCAGGGCTTCC TTTCCTCTGCCATCAATGATGGTGATGATGGTG**CGTGCTCAGTCTGGGCC – 3' (SEQ ID NO: 773)

3'-MS2_13nt-PBS:

5'**GGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGTGATGATGGT G**CGTGCTCAGTCTG**AAATTAACAAATCAA**GCCAACATGAGGATCACCCATGTCT GCAGGGCC – 3' (SEQ ID NO: 774)

3'-MS2_17nt-PBS:

5'**GGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCAATGATGGTGATGATGGT G**CGTGCTCAGTCTGGGCC**AAATTAACAAATCAA**GCCAACATGAGGATCACCCAT GTCTGCAGGGCC – 3' (SEQ ID NO: 775)

Sequences of MS2 PEgRNAs:

Spacer / MS2 aptamer / *sgRNA scaffold* / **RT template** / PBS

HEK3_MS2_1
5'**GGCCCAGACTGAGCACGTGA***GTTTTAGAGCTAG*GCCAACATGAGGATCACCCA TGTCTGCAGGGCC*TAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTG GGACCGAGTCGGTCC***TCTGCCATCT**CGTGCTCAGTCT – 3' (SEQ ID NO: 776)

HEK3_MS2_2
5'**GGCCCAGACTGAGCACGTGA***GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC TAGTCCGTTATCAACTTG*GCCAACATGAGGATCACCCATGTCTGCAGGGCC*AAGTG GGACCGAGTCGGTCC***TCTGCCATCT**CGTGCTCAGTCT – 3' (SEQ ID NO: 777)

Protein sequences:

MS2cp-*PE2*
MKRTADGSEFESPKKKRKVGSASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISS NSRSQAYKVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGEELPVAGWRSYLNMELT

IPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSS*DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALL*
FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKH
ERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLN
PDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGL
FGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLS
DAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGY
AGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELH
AILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVV
DKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSG
EQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDK
DFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIA
NLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIE
EGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSF
LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERG
GLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRK
DFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEI
GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMP
QVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRM
LASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQIS
EFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRY
TSTKEVLDATLIHQSITGLYETRIDLSQLGGDSGGSSGGSSGSETPGTSESATPESSGGSSGG
SSTLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVS
IKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNK
RVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGIS
GQLTWTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGT
RALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQ
LREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGL
PDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAV
LTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVA
LNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKA
GAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIY
RRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARK
*AAITETPDTSTLLIENSSP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 778)

MS2cp-*MMLV-RT*
MKRTADGSEFESPKKKRKVGSASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISS
NSRSQAYKVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGEELPVAGWRSYLNMELT
IPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYSGGSSGGSSGSETPGTSESATP
ESSGGSSGGSS*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLII*
PLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRP
VQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFE
WRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMG
QPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQ
ALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPP
CLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTD

*RVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSL LQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFA TAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRM ADQAARKAAITETPDTSTLLIENSSP*SGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 779)

*MMLV-RT-*MS2cp
MKRTADGSEFESPKKKRKV*TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGM GLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPV KKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLH PTSQPLFAFEWRDPEMGISGQLTWTRLPQGFKNSPTLFDEALHRDLADFRIQHPDLILLQ YVDDLLLAATSELDCQQGTRALLQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLT EARKETVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQ QKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLD PVAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTH YQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADH TWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKG HSAEARGNRMADQAARKAAITETPDTSTLLIENSSP*SGGSSGGSSGSETPGTSESATPESS GGSSGGSSGSASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNSRSQAYKVTCS VRQSSAQNRKYTIKVEVPKVATQTVGGEELPVAGWRSYLNMELTIPIFATNSDCELIV KAMQGLLKDGNPIPSAIAANSGIYSGGSKRTADGSEFEPKKKRKV (SEQ ID NO: 780)

EXAMPLE 20. SPLIT-INTEIN DELIVERY OF PRIME EDITORS

[1522] This Example demonstrates that a prime editor may be split using inteins as a means to deliver the prime editor to cells via separate vectors, wherein each vector encodes a portion of the prime editor fusion protein. This Example is focused on a PE fusion protein comprising the canonical SpCas9 (SEQ ID NO: 18). The split sites for other Cas9 proteins may be the corresponding same site, or may need to be optimized for each different Cas9 protein. In the instant Example, the prime editor was split between residues 1023 and 1024 of SpCas9 (SEQ ID NO: 18). This is referred to as the "1023/1024" split site.

[1523] Prime editors (PEs) exceed the AAV packaging capacity. It was therefore contemplated to split the PE by inserting a trans-splicing *Npu* intein at *S. pyogenes* Cas9 residue 1024 in SEQ ID NO: 18, allowing delivery of split-SpPEs as two separate polypeptides, each encoded by one of a dual-AAV system. The split site 1023/1024 was chosen because it (1) allows packaging into two AAVs while accommodating space for guide cassette(s) and minimal regulatory elements, (2) mutation of the native Serine to Cysteine would be relatively conservative, (3) the site is a flexible loop near the periphery of Cas9 which sterically is predicted to allow for spicing to occur, and (4) Cas9 has been successfully altered in this loop by circular permutation (but not previously at this specific split site).

[1524] To determine whether *Npu*-split prime editors are active, HEK cells were transfected with plasmids encoding split-editors, finding they recapitulate activity of full-length PE3 as analyzed by high-throughput sequencing. Furthermore, the three native *Npu* amino-terminal residues of C-terminal extein are known to splice most efficiently but differ from those natively flanking Cas9 residue 1024. Replacement of these Cas9 residues with the native *Npu* residues may alter the activity of prime editor. It was therefore determined whether mutation from the Cas9-native "SEQ" towards the *Npu* intein "CFN" sequence altered efficiency of prime editing. The "SEQ" residues facilitate prime editing with similar efficiency to full-length, suggesting the intein-split PE halves are able to associate and mediate prime editing. No further increase was seen by mutation towards "CFN", suggesting that association alone may be sufficient for split-PE activity, as we have previously observed with intein-split base editors.

[1525] Although associated, unspliced editors may be active, perturbations of the sterics of the prime editor resulting from incomplete splicing during initiation steps may affect editing outcomes. 1024-CFN was therefore chosen for further studies as it also recapitulates full-length PE3 activity.

[1526] The following are the amino acid sequences of the 1023/1024 split.

[1527] SpPE2 split at 1023/1024 N terminal half

[1528] MKRTADGSEFESPKKKRKVDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKV
LGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAK
VDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDA
KAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS
KDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRY
DEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEK
MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKI
EKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNF
DKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFK
TNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEEN
EDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANL
AGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKR
IEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDA

IVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRK FDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREV KVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVY GDYKVYDVRKMIAK*CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPVAQW HDRGEQEVFEYCLEDGSLIRATKDHKFMTVDGQMLPIDEIFERELDLMRVDNLPN***SGGS*** *KRTADGSEFEPKKKRKV* (SEQ ID NO: 3875)

[1529] Key: NLS, Cas9, Mutated residues, **Linker**, *NpuN intein*, *NpuC intein*, **RT**

[1530] SpPE2 split at 1023/1024 C terminal half

[1531] MKRTADGSEFESPKKKRKV*IKIATRKYLGKQNVYDIGVERDHNFALKNGFIASN*C FNEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVR KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAY SVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLP KYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK QLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFT LTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD***SGGSS GGSSGSETPGTSESATPESSGGSSGGSS*TLNIEDEYRLHETSKEPDVSLGSTWLSD FPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQYPMSQEARLGIKPHIQRLL DQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVEDIHPTVPNPYNLL SGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLP QGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRAL LQTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPK TPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIK QALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDP VAAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLS NARMTHYQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTR PDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQ RAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTSEGKEIK NKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPD TSTLLIENSSP***SGGS****KRTADGSEFEPKKKRK* SEQ ID NO: 3876)

[1532] Key: NLS, Cas9, Mutated residues, **Linker**, *NpuN intein*, *NpuC intein*, **RT**

Prime editor packages in a dual AAV system

[1533] Prime editors split between residues 1023 and 1024 with Npu trans-splicing intein become packages within AAV with high titer comparable to that of base editors of similar genome size produced in the same manner.

Prime editing in vivo

[1534] Split SpPE3 delivered with AAV9 by intracerebroventricular injection to P0 mice mediates prime editing in brain tissue *in vivo*. A nucleotide substitution at position +5 (i.e., 5 nucleotide bases downstream of nick site and within the PAM sequence) of a G-to-T results in the installation of a Pro>Gln coding mutation near the N terminus of DNMT1. This edit demonstrates the viability of prime editing *in vivo* and is not thought to introduce any selective pressure on edited cells (with "+5" referring to the +5 position downstream of the nick site). The AAV architectures tested include a full-length MMLV RT as well as a truncated variant lacking the RNAse H domain. The truncated post-transcriptional regulatory element W3 was also assessed as it has been shown to increase expression from viral cassettes in vivo but its importance had not been tested in the context of base editors or prime editors. It was found that full-length RT outperforms truncated RT, and the addition of the W3 sequence improves activity. The increase in editing activity when W3 is present indicates that expression of prime editor is limiting *in vivo* and demonstrates the distinct challenges of prime editing *in vivo* versus cell culture.

References

[1535] Oakes, B.L., Fellmann, C. et al. CRISPR-Cas9 Circular Permutants as Programmable Scaffolds for Genome Modification. Cell. 2019 Jan 10;176(1-2):254-267.e16. doi: 10.1016/j.cell.2018.11.052.

EXAMPLE 21. LINKER OPTIMIZATION IN PE2 FORMAT

[1536] This Example constructed a number of variant prime editors all based on PE2. PE2 has the following sequence and structure:

[1537] As used herein, "PE2" refers to a PE complex comprising a fusion protein comprising Cas9(H840A) and a variant MMLV RT having the following structure: [NLS]-[Cas9(H840A)]-[linker]-[MMLV_RT(D200N)(T330P)(L603W)(T306K)(W313F)] + a

desired PEgRNA, wherein the PE fusion has the amino acid sequence of SEQ ID NO: 134, which is shown as follows:

MKRTADGSEFESPKKKRKV**DKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGN**
TDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAK
VDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTD
KADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPIN
ASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFD
LAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEI
TKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHA
ILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYV
TEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVE
DRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYA
HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELV
KVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPV
ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQSFLKDDSIDNK
VLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGL
SELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLV
SDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYD
VRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIV
WDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDW
DPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDF
LEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNF
LYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKV
LSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT
LIHQSITGLYETRIDLSQLGGD<u>**SGGSSGGSSGSETPGTSESATPESSGGSSGGSS**</u>*TL*
NIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIIPLKATSTPVSIKQY
PMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNKRVED
IHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGISGQLT
WTRLPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQGTRALL
QTLGNLGYRASAKKAQICQKQVKYLGYLLKEGQRWLTEARKETVMGQPTPKTPRQLREF
LGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAPALGLPDLTK
PFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRMVAAIAVLTKDAG
KLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPVVALNPATLL
PLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSLLQEGQRKAGAAVTTET
EVIWAKALPAGTSAQRAELIALTQALKMAEGKKLNVYTDSRYAFATAHIHGEIYRRRGWLTS
EGKEIKNKDEILALLKALFLPKRLSIIHCPGHQKGHSAEARGNRMADQAARKAAITETPDT
STLLIENSSP<u>SGGSKRTADGSEFEPKKKRKV</u> (SEQ ID NO: 134)

KEY:
<u>NUCLEAR LOCALIZATION SEQUENCE (NLS)</u> TOP:(SEQ ID NO: 124), BOTTOM: (SEQ ID NO: 133)
CAS9(H840A) (SEQ ID NO: 137)
<u>33-AMINO ACID LINKER</u> (SEQ ID NO: 127)

[1538] M-MLV reverse transcriptase (SEQ ID NO: 139).

[1539] The PE2 linker is SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127).

[1540] In this experiment, the linker of PE2 was replaced with one of the following substitute linkers (or no linker in one instance):

Linker Name	Nucleotide Sequence	Amino Acid Sequence
1x SGGS	TCCGGAGGATCT (SEQ ID NO: 3880)	SGGS (SEQ ID NO: 3887)
2x SGGS	TCCGGAGGATCTAGCGGAGGCTCC (SEQ ID NO: 3881)	SGGSSGGS (SEQ ID NO: 3888)
3x SGGS	TCCGGAGGATCTAGCGGAGGCTCCA GCGGAGGCAGC (SEQ ID NO: 3882)	SGGSSGGSSGGS (SEQ ID NO: 3889)
1x XTEN	TCTGGCTCTGAGACACCTGGCACAAG CGAGAGCGCAACACCTGAAAGC (SEQ ID NO: 3883)	SGSETPGTSESATPES (SEQ ID NO: 3890)
No linker		
1x Gly	GGT	G
1x Pro	CCC	P
1x EAAAK	GAAGCAGCTGCTAAA (SEQ ID NO: 3884)	EAAAK (SEQ ID NO: 3891)
2x EAAAK	GAAGCCGCTGCTAAAGAAGCTGCAG CTAAG (SEQ ID NO: 3885)	EAAAKEAAAK (SEQ ID NO: 3892)
3x EAAAK	GAAGCCGCTGCTAAAGAGGCCGCTG CTAAAGAAGCTGCAGCTAAG (SEQ ID NO: 3886)	EAAAKEAAAKEAAAK (SEQ ID NO: 3893)

[1541] FIG. 79 shows the editing efficiency of the replacement linker constructs. In particular, the data shows the editing efficiency of the PE2 construct with the current linker (noted as PE2 – white box) compared to various versions with the linker replaced with a sequence as indicated at the HEK3, EMX1, FANCF, RNF2 loci for representative PEgRNAs for transition, transversion, insertion, and deletion edits. The replacement linkers are referred to as “1x SGGS”, “2x SGGS”, “3x SGGS”, “1x XTEN”, “no linker”, “1x Gly”, “1x Pro”, “1x EAAAK”, “2x EAAAK”, and “3x EAAAK”. The editing efficiency is measured in bar graph format relative to the “control” editing efficienty of PE2. The linker of PE2 is SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 127). All editing was done in the context of the PE3 system, i.e., which refers the PE2 editing construct plus the addition of the optimal secondary sgRNA nicking guide.

[1542] FIG. 80. shows that the 1x XTEN linker provided an increase in editing efficiency. Taking the average fold efficacy relative to PE2 yields the graph shown, indicating that use of a 1x XTEN linker sequence improves editing efficiency by 1.14 fold on average (n=15).

EXAMPLE 22. PRIME EDITOR GUIDE RNAs WITH IMPROVED ACTIVITIES

[1543] In various embodiments, PEgRNA are likely to be target DNA site and edit dependent. That is, the sequence of the PEgRNA will depend upon the target DNA sequence and the particular edit that is being introduced therein by prime editing (e.g., deletion, insertion, inversion, substitution). For instance, in certain embodiments, when attaching a motif 3' of the primer binding site (PBS) of the PEgRNA, a linker between the PBS and motif is preferred to prevent steric clash with the polymerase domain (e.g., reverse transcriptase) of the PE fusion protein. However, the nature of that linker may be different for each site. For instance, if the same linker for each site were used, it could artificially render a 13 nt PBS a 16 nt PBS by fortuitous pairing to the spacer sequence. Similarly, the linker could basepair to the PBS itself, resulting in its occlusion and potentially reducing activity. So, unlike with protein-based editors, such as PE or BE4, a single linker sequence option connecting two elements may not be effective in each construc but will depend in part on the sequence of the target DNA sequence and the edit of interest.

[1544] Building, in part, on the information presented Example 15 (Design and Engineering of PEgRNAs), this Example constructed and then tested the effects of various structural modifications made to PEgRNA on editing function, among other aspects.

Expression of PEgRNAs from non-pol III promoters

[1545] A variety of PEgRNA expression systems were tested for their ability to generate PEgRNAs, using insertion of a 102 nucleotide sequence from FKBP as a readout.

[1546] Transcription of PEgRNA can be directed by a typical constitutive promoter, such as U6 promoter. Although the U6 promoter is in most cases effective at directing transcription of PEgRNAs, the U6 promoter is not very effective at directing the transcription of longer PEgRNAs or U-rich RNAs. U-rich RNA stretches of cause premature termination of transcription. This Example compared editing outcomes of guides expressed from the CMV promoter or U1 promoter with the U6 promoter. These promoters require a different terminator sequence, such as MASC ENE or PAN ENE, as provided below. An increase in editing was observed with the pCMV/MASC-ENE system, however these guides resulted in incomplete insertion of the sequence, while, with the U6 promoter, complete insertion was observed at lower levels of editing. See FIG. 81. The data suggests the likelihood that the alternate expression systems may be useful for long insertions.

[1547] The nucleotide sequence of the pCMV/MASC-ENE expression systems as follows (5'-to-3' direction) (with the name of the motif in bold immediately preceding the region to which it refers):

[1548] -**pCMV promoter**-

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAG
TTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACC
CCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGAC
TTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTA
CATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT
GGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCA
GTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTAC
ATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA
TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATG
TCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGA
GGTCTATATAAGCAGAGCTGGTTTAGTGAACCGTCAGATC-**Csy4 loop**-
GTTCACTGCCGTATAGGCAG-**spacer**-GGCCCAGACTGAGCACGTGA-**scaffold**-
GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA
AAAGTGGGACCGAGTCGGTCC-**template**-
TGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCA-**insert**-
AAATTTCTTTCCATCTTCAAGCATCCCGGTGTAGTGCACCACGCAGGTCTGGCCG
CGCTTGGGGAAGGTGCGCCCGTCTCCTGGGGAGATGGTTTCCACCTGCACTCC-
PBS-CGTGCTCAGTCTG-**linker**-TTT-**MASC ENE**-
TAGGGTCATGAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTTCTCAGG
TTTTGCTTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGCAAAAGATGCTGGT
GGTTGGCACTCCTGGTTTCCAGGACGGGGTTCAAATCCCTGCGGCGTCTTTGCTTT
GACT-**unrelated plasmid sequence**-
TTTTTTTAAGCTTGGGCCGCTCGAGGTAGCAGC-**Ubc promoter**-
GGCCTCCGCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTCACGGCGAG
CGCTGCCACGTCAGACGAAGGGCGCAGGAGCGTTCCTGATCCTTCCGCCCGGAC
GCTCAGGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCA
GCAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTTCTTT
CCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCTCGGCGATTCTGCGGAG
GGATCTCCGTGGGGCGGTGAACGCCGATGATTATATAAGGACGCGCCGGGTGTG

GCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGTTCTTGTTTGTGGATC
GCTGTGATCGTCACTTGGTGAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTG
GCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGGCTGT
AGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGGGGTTGGGGGGAGCGCACA
AAATGGCGGCTGTTCCCGAGTCTTGAATGGAAGACGCTTGTAAGGCGGGCTGTG
AGGTCGTTGAAACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAGGTCTTG
AGGCCTTCGCTAATGCGGGAAAGCTCTTATTCGGGTGAGATGGGCTGGGGCACC
ATCTGGGGACCCTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGGTTTGTCGT
CTGGTTGCGGGGGCGGCAGTTATGCGGTGCCGTTGGGCAGTGCACCCGTACCTTT
GGGAGCGCGCGCCTCGTCGTGTCGTGACGTCACCCGTTCTGTTGGCTTATAATGC
AGGGTGGGGCCACCTGCCGGTAGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACG
CAGGGTTCGGGCCTAGGGTAGGCTCTCCTGAATCGACAGGCGCCGGACCTCTGGT
GAGGGGAGGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTTATGTACCTATCT
TCTTAAGTAGCTGAAGCTCCGGTTTTGAACTATGCGCTCGGGGTTGGCGAGTGTG
TTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGTAATCATTTGGGTCAATATGTAA
TTTTCAGTGTTAGACTAGTAAATTGTCCGCTAAATTCTGGCCGTTTTTGGCTTTTT
TGTTAGACAGGATCCCCGGGTACCGGTCGCCACC-**Csy4 and NLS**-
ATGGGCTCTTTTACTATGGACCACTACCTGGATATTAGACTGAGACCTGACCCTG
AGTTCCCACCCGCCCAGCTGATGAGCGTGCTGTTCGGCAAGCTGCACCAGGCCCT
GGTGGCACAGGGAGGCGACCGGATCGGCGTGAGCTTCCCCGACCTGGATGAGAG
CAGATCCAGGCTGGGAGAGCGCCTGAGGATCCACGCATCCGCCGACGATCTGCG
CGCCCTGCTGGCCCGGCCATGGCTGGAGGGCCTGCGCGACCACCTGCAGTTTGG
AGAGCCAGCAGTGGTGCCACACCCTACCCCATACAGGCAGGTGTCCAGGGTGCA
GGCAAAGTCTAACCCTGAGCGGCTGCGGAGAAGGCTGATGCGCCGGCACGATCT
GTCTGAGGAGGAGGCCAGAAAGAGGATCCCCGACACCGTGGCCAGAACACTGG
ATCTGCCTTTCGTGACCCTGCGGAGCCAGAGCACAGGCCAGCACTTCAGACTGTT
TATCAGGCACGGCCCACTGCAGGTGACAGCCGAGGAAGGAGGATTCACTTGTTA
CGGACTGTCTAAAGGAGGATTCGTGCCCTGGTTCAGCAGCCTGAGGCCTCCTAAG
AAGAAGAGGAAGGTTTAA-**SV40 terminator**-
TGATCATAATCAAGCCATATCACATCTGTAGAGGTTTACTTGCTTTAAAAAACCT
CCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACT
TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCAC

AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATG TATCTTATCATGTCTGGATCTGC.

Key:

[pCMV promoter] – binds pol II RNA polymerase

[Csy4 loop] – bound by Csy4 protein, results in cleavage 3' of the loop. Required because part of [CMV promoter] is transcribed, and if this sequence is attached 5' of the gRNA it will lower/eliminate activity (previously known).

[Spacer sequence] of pegRNA

[pegRNA scaffold]

[DNA synthesis template]

[insertion edit (108 nt from FKBP)]

[primer binding site]

[Linker] (highly variable) – connects PBS and terminator element

[MASC ENE transcription terminator] - transcription of this element results in termination of transcription; a polyA tail is encoded and then sequestered by the ENE element

[Unimportant sequence]

[Ubc promoter] – required for expression of the Csy4 protein

[Csy4 protein and NLS] – required for processing of the 5' end of the guide. Other strategies could also be used that don't require expression of a large protein (such as ribozyme-mediated cleavage of the spacer), but these would require more individual tuning for different spacer sequences, so we used this.

[SV40 terminator] – for termination of the Csy4 protein.

Improvements to the PEgRNA scaffold

[1549] A number of structural modifications to the gRNA scaffold were also tested, none of which showed a significant increase in editing activity (see FIG. 82 at 3.30.13 through 3.30.19 inin the X axis, as compare to 3.30). However, this data has two caveats worth noting. First, this guide already worked quite well, and a less effective guide would have been better to test. Second, in HEK cells, transfection is quite efficient, and it was noted that the amount of guide RNA transfected is in large excess compared to what is needed (reducing the amount by ~4-8 fold has no effect on editing). These improvements might only be seen in other cell types, where transfection efficiency is lower, or with less effective guides. Many of these changes are precedented to improve sgRNA activity in other cell lines.

[1550] The sequences of the constructs of FIG. 82 are as follows:

HEK3.30 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Templateand PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3873]
HEK3.30.0 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUUUU [SEQ ID NO: 3874]
HEK3.30.1 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-[none] [SEQ ID NO: 3875]
HEK3.30.2 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGCUCGAGGCGGAAACGCCUCGAGCUUUU [SEQ ID NO: 3876]
HEK3.30.2b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGCUCGAGGCGGAAACGCCUCGAGC [SEQ ID NO: 3877]
HEK3.30.3 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGCUCGAGGCGUACGCGAAAGCGUACGCCUCGAGCUUUU [SEQ ID NO: 3878]
HEK3.30.3b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGCUCGAGGCGUACGCGAAAGCGUACGCCUCGAGC [SEQ ID NO: 3879]
HEK3.30.5 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGCUCGAGGCGUACGCCCGAUGAAAAUCGGGCGUACGCCUCGAGCUUUU [SEQ ID NO: 3880]
HEK3.30.5a pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUUGGGGUUGGGGUUGGGGUUGGGGUUUU [SEQ ID NO: 3881]
HEK3.30.5b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU

UGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUGGUGGUGGUGGUUUU [SEQ ID NO: 3882]
HEK3.30.13 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGCGAAAGCUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3883]
HEK3.30.15 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGCUCGAAAGAGCUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3884]
HEK3.30.15 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGCUCAUGAAAAUGAGCUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3885]
HEK3.30.16 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGCUCAUCCGAAAGGAUGAGCUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3886]
HEK3.30.17 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGCUCAUCCUGGAAACAGGAUGAGCUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3887]
HEK3.30.18 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUGAGAGCUAGAAAUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3888]
HEK3.30.19 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUGAGAGCUAGCUCAUGAAAAUGAGCUAGCAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3889]
HEK3.56 pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAAAGCUUCGACCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3890]
HEK3.56.1a pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGGCGAAAGCCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3891]
HEK3.56.1b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGACGAAAGCCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3892]

HEK3.56.1c pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGGCGAAAGCCCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3892]
HEK3.56.2a pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGAUGCGAAAGCAUCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3893]
HEK3.56.2b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGAUGCGAAAGCACCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3894]
HEK3.56.2c pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGAUGCGAAAGCAUCCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3895]
HEK3.56.3a pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGACAUGCGAAAGCAUGUCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3896]
HEK3.56.3b pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGACAUGCGAAAGCAGGCCCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3897]
HEK3.56.3c pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGC-Template and PBS-UCUGCCAUCAGACAUGCGAAAGCAUGUCUCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3898]
HEK3.56.4a pegRNA sequence: spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAUACGUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUUACGAAGUGGGACCGAGUCGGUCC-Template and PBS-UCUGCCAUCAAAGCUUCGACCGUGCUCAGUCUG-Terminal motif-UUUU [SEQ ID NO: 3899]
HEK3.56.4b pegRNA sequence: 5'motif-GCAGACCUAAGUGGUGACAUAUGGUCUG-spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAUACGUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUUACGAAGUGGGACCGAGUCGGUCC-Template and PBS--Terminal motif-UUUU [SEQ ID NO: 3922]
HEK3.56.4c pegRNA sequence: 5'motif-GCAGACCUAAGUGGUGACAUAUGGUCUG-spacer-GGCCCAGACUGAGCACGUGA-scaffold-

GUUUUAGAGCUAUACGUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UUACGAAGUGGGACCGAGUCGGUCC-Template and PBS--Terminal motif-UUUU [SEQ ID NO: 3900]
HEK3.56.4d pegRNA sequence: 5'motif-GCAGACCUAAGUGGUGACAUAUGGUCUG-spacer-GGCCCAGACUGAGCACGUGA-scaffold-GUUUUAGAGCUAUACGUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACU UUACGAAGUGGGACCGAGUCGGUCC-Template and PBS--Terminal motif-UUUU [SEQ ID NO: 3901]

Note that where ever either no terminal motif or a terminal motif that does not end in a run of U's exists, transcript was terminated using the following HDV ribozyme: GGCCGGCAUGGUCCCAGCCUCCUCGCUGGCGCCGGCUGGGCAACAUGCUUCGG CAUGGCGAAUGGGAC [SEQ ID NO: 3923]

Additional RNA motifs

[1551] See FIG. 82 for details on certain motifs, such as an HDV ribozyme 3' of the PEgRNA, or G-quadruplex insertion, P1 extensions, template hairpins, and tetraloop circ'd, that may be introduced into a PEgRNA to improve its performance.

[1552] In particular, this Example tested the effect of intalling a tRNA motif 3' of the primer binding site. This element was chosen because of multiple potential functions:

[1553] (1) the tRNA motif is a very stable RNA motif, and so could potentially reduce PEgRNA degradation;

[1554] (2) the MMLV RT uses a prolyl-tRNA as a primer when converting the viral genome into DNA during transcription, so it was suspected the same cap could be bound by the RT, improving binding of the PEgRNA by PE, RNA stability, and bringing the PBS back in closer proximity to the genomic site, potentially also improving activity.

[1555] In these constructs, the P1 of the tRNA (see FIG. 84) was extended. P1 refers to the first stem/base-pairing element of the tRNA (see FIG. 84). This was believed to be necessary to prevent RNAseP-mediated cleavage of the tRNA 5' of the P1, which would result in its removal from the PEgRNA.

[1556] In this design a prolyl-tRNA (codon CGG) with an extended P1 and short 3 nt linker between the tRNA and the PBS was used. A variety of tRNA designs were tested and the editign efficiency was tested compared to a PEgRNA having no tRNA cap – see the comparative data in FIG. 83 (depicting a PE experiment that targeted editing of the HEK3 gene, specifically targeting the insertion of a 10 nt insertion at position +1 relative to the nick

site and using PE3), FIG. 85 (depicting a PE experiment that targeted editing of the FANCF gene, specifically targeting a G-to-T conversion at position +5 relative to the nick site and using PE3 construct) and FIG. 86 (depicting a PE experiment that targeted editing of the HEK3 gene, specifically targeting the insertion of a 71 nt FLAG tag insertion at position +1 relative to the nick site and using PE3 construct). tRNA-modified PEgRNAs were tested against a non-modified PEgRNA control.

[1557] UGG/CGG refers to the codon used, the number refers to the length of the added P1 extension, long indicates an 8 nt linker, no designation a 3 nt linker.

[1558] The data suggest that the installation of a tRNA may enable use of shorter PBSs, which would likely result in additional activity improvements. In the case of RNF2, it is possible/likely that the linker used resulted in improved PBS binding to the spacer, and the resulting diminishment in activity.

[1559] Some sequences used:

[1560] HEK3 +1 FLAG-tag insertion, proly-tRNA{UGG} P1 ext 5 nt, linker 3 nt

[1561] GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGA GGAAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAU CCGUGCUCAGUCUGUCUGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCU UCGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3902]

[1562] FANCF +5 G to T proly-tRNA{CGG} P1 ext 5 nt, linker 3 nt

[1563] GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAA AAGCGAUCAAGGUGCUGCAGAAGGGAUCUGGCGGGGCUCGUUGGUCUAGGGG UAUGAUUCUCGCUUCGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCC CCGCCUUUU [SEQ ID NO: 3903]

[1564] HEK3 ++1 10 nt insertion, proly-tRNA{UGG} P1 ext 5 nt, linker 3 nt

[1565] GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGGACCGAGUCGGUCCUCUG CCAUCAAAGCUUCGACCGUGCUCAGUCUUCUGCUCGAGGCGGGGCUCGUUGGU CUAGGGGUAUGAUUCUCGCUUCGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGG ACGAGCCCCGCCUCGAGCUUUU [SEQ ID NO: 3904]

[1566] The sequences reported in the data of FIG. 85 and 86 are as follows:

FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-partial CGG tRNA linker 8-UCUCUCUCUGGUCUAGGGGUAUGAUUCUCGCUUCGGGUGCGAGAGGUCCCGGGUUCAAUUUU [SEQ ID NO: 3905]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 5 linker 3-UCUGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3906]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 5 linker 8-UCUCUCUCGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3907]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 8 linker 3-UCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3908]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 8 linker 8-UCUCUCUCCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3909]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 11 linker 3-UCUGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUUUU [SEQ ID NO: 3910]
FANCF +5 G to T pegRNA sequence: space, scaffold template and PBSr-GGAAUCCCUUCUGCAGCACCGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGGAAAAGCGAUCAAGGUGCUGCAGAAGGGA-UGG P1 ext 11 linker 8-UCUCUCUCGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUU

UGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUU UU [SEQ ID NO: 3911]
HEK3 +1 10 nt insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAG GCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTCTGCCATCA AAGCTTCGACCGTGCTCAGTCTG-UGG P1 ext 5 linker 3-UCUGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGA GGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3913
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGAGG AAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAUC CGUGCUCAGUCUG-partial CGG tRNA linker 8-UCUCUCUCUGGUCUAGGGGUAUGAUUCUCGCUUCGGGUGCGAGAGGUCCCG GGUUCAAUUUU [SEQ ID NO: 3914]
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGAGG AAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAUC CGUGCUCAGUCUG-UGG P1 ext 5 linker 3-UCUGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGA GGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3915]
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGAGG AAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAUC CGUGCUCAGUCUG-UGG P1 ext 5 linker 8-UCUCUCUCGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUG CGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUUUU [SEQ ID NO: 3916]
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAA UAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGG AGGAAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUG UAAUCCGUGCUCAGUCUG-UGG P1 ext 8 linker 8-UCUCUCUCCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUG GGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3917]
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGAGG AAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAUC CGUGCUCAGUCUG-UGG P1 ext 11 linker 8-UCUCUCUCGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUU UGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUU UU [SEQ ID NO: 3918]
HEK3 +1 FLAG insertion pegRNA sequence: space, scaffold template and PBSr-GGCCCAGACUGAGCACGUGAGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA

AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUGGAGG AAGCAGGGCUUCCUUUCCUCUGCCAUCACUUAUCGUCGUCAUCCUUGUAAUC CGUGCUCAGUCUG-UGG P1 ext 14 linker 8- UCUCUCUCGGUGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCG CUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAG CACCUUUU [SEQ ID NO: 3919]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{CGG}-5- UCUGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUCGGGUGCGAGA GGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCC [SEQ ID NO: 3920]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{CGG}-8- UCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUCGGGUGCG AGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3921]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{CGG}-11- UCUGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUCGGGU GCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUUUU [SEQ ID NO: 3922]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{Lys}-5- UCUGGCGGGCCCGGAUAGCUCAGUCGGUAGAGCAUCAGACUUUUAAUCUGA GGGUCCAGGGUUCAAGUCCCUGUUCGGGCCCGCCUUUU [SEQ ID NO: 3923]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{Lys}-8- UCUCGAGGCGGGCCCGGAUAGCUCAGUCGGUAGAGCAUCAGACUUUUAAUC UGAGGGUCCAGGGUUCAAGUCCCUGUUCGGGCCCGCCUCGUUUU [SEQ ID NO: 3924]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC CUCAUGUAAUGACUAAGAUG-tRNA-Pro{UGG}-8- UCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCG AGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3925]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr- GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUA AGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACAC

CUCAUGUAAUGACUAAGAUG-tRNA-Pro{UGG}-11-UCUGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUUUU [SEQ ID NO: 3926]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr-GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACACCUCAUGUAAUGACUAAGAUG-tRNA-Pro{UGG}-8-longer linker-UCUCUCUCCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGUUUU [SEQ ID NO: 3927]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr-GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACACCUCAUGUAAUGACUAAGAUG-tRNA-Pro{UGG}-11-longer linker-UCUCUCUCGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCUUUU [SEQ ID NO: 3928]
RNF2 +1 C to A pegRNA sequence: space, scaffold template and PBSr-GUCAUCUUAGUCAUUACCUGGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCGAACACCUCAUGUAAUGACUAAGAUG-tRNA-Pro{UGG}-14-longer linker-UCUCUCUCGGUGCUCGAGGCGGGGCUCGUUGGUCUAGGGGUAUGAUUCUCGCUUUGGGUGCGAGAGGUCCCGGGUUCAAAUCCCGGACGAGCCCCGCCUCGAGCACCUUUU [SEQ ID NO: 3929]

EXAMPLE 23. USE OF PRIME EDITING TO CORRECT CDKL5 and SICKLE CELL ANEMIA

Use of PE to design mouse model of CDKL5 with 1412delA mutation

[1567] CDKL5 Deficiency Disorder (CDD) is a neurodegenerative disease most often caused by spontaneous mutations in the cyclin-dependent kinase-like 5 gene. Symptoms manifest in early childhood and include seizures, irregular sleeping patterns, gastrointestinal stress, and developmental delay. Some mutations which cause CDD, including 1412delA, cannot be corrected with base editing. However, prime editing has the potential to precisely correct all base-to-base changes, deletions, and insertions. With focus on the 1412delA mutation, this Example designed and tested pegRNAs capable of inserting the mutation in hopes to establish a mouse neuronal cell line (N2A) harboring the mutation. This will allow for extensive screening potentially therapeutic pegRNAs that correct the mutation. The ultimate goal is to be able to move into a CDD mouse model with the 1412delA mutation to assess therapeutic effect. No current mouse models of CDD have a humanized allele, however

optimization of pegRNAs is underway in HEK293T cells as well. FIGs. 87 and 88 are the results from a pilot screen in N2A cells where the pegRNA installs 1412Adel, with details about the primer binding site (PBS) length and reverse transcriptase (RT) template length. (Shown with and without indels)

Use of PE to treat sickle cell anemia (SCA)

[1568] Sickle cell anemia (SCA) is a recessive blood disorder caused by a glutamate-to-valine mutation at position 6 in the β-globin gene. The result is sickled red blood cells that are poor oxygen transporters and prone to aggregation. Symptoms of aggregation can be life-threatening. Previously, the D. Liu lab was able to show both the installation and correction of the SCA locus in HEK293T cells using prime editing via DNA plasmid transfection. Since hematopoietic stem cells (HSCs) are difficult to edit via DNA plasmid transfection, this Example tested the PE3 system in HSCs with protein and mRNA nucleofection. FIG. 89 are the results of editing at a proxy locus in the β-globin gene and at HEK3 in healthy HSCs, varying the concentration of editor to pegRNA and nicking gRNA.

mRNA nucleofection protocol:

[1569] The protocol was improved by adjusting the ratio of editor to guides ([editor] to [guide] ratio or editor:guide ratio)

[1570] The nicking guide protospacer sequence was: CCTTGATACCAACCTGCCCA

[1571] The pegRNA sequence was:
CATGGTGCACCTGACTCCTGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
TAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAGACTTCTCTTCAG
GAGTCAGGTGCACTTT

[1572] 1. Thaw CD34+ cells and pre-stimulate in X-Vivo15 media with cytokines (SCF, Flt3 and TPO) for 24 hrs. Seeding density 1 million cells/mL.

[1573] 2. Next day (post 24 hrs), aspirate CD34+ cells (1 x 105) and wash once with PBS (centrifugation performed at 300g for 10 mins in RT) and resuspend in P3 solution (Lonza) (20 ul per condition).

[1574] 3. In hood, combine 2 ug of SpPE2 mRNA, 1ug combined of pegRNA and sgRNA in a sterile PCR strip on ice. Adjust the volume to 20 ul (cells in P3 solution + mRNA &gRNA mix).

[1575] 4. Pipette up cell suspension into 20 uL Lonza4D 16 well strip and electroporate with program DS130.

[1576] 5. Wait for 10 to 15 mins post electrporation and add 80 uL of X-Vivo10+cytokine media to cell suspension and transfer to pre-warmed X-Vivo10 with cytokine media.

[1577] 6. Harvest cells 72 hours after electroporation and check the cell recovery and do genotyping in bulk population and sorted CD34+ and CD34+90+ populations as well.

Wildtype CDKL5 protein (Accession No. NP_001032420) (isoform 1 – human)

MKIPNIGNVM NKFEILGVVG EGAYGVVLKC RHKETHEIVA IKKFKDSEEN
EEVKETTLRE LKMLRTLKQE NIVELKEAFR RRGKLYLVFE YVEKNMLELL
EEMPNGVPPE KVKSYIYQLI KAIHWCHKND IVHRDIKPEN LLISHNDVLK
LCDFGFARNL SEGNNANYTE YVATRWYRSP ELLLGAPYGK SVDMWSVGCI
LGELSDGQPL FPGESEIDQL FTIQKVLGPL PSEQMKLFYS NPRFHGLRFP
AVNHPQSLER RYLGILNSVL LDLMKNLLKL DPADRYLTEQ CLNHPTFQTQ
RLLDRSPSRS AKRKPYHVES STLSNRNQAG KSTALQSHHR SNSKDIQNLS
VGLPRADEGL PANESFLNGN LAGASLSPLH TKTYQASSQP GSTSKDLTNN
NIPHLLSPKE AKSKTEFDFN IDPKPSEGPG TKYLKSNSRS QQNRHSFMES
SQSKAGTLQP NEKQSRHSYI DTIPQSSRSP SYRTKAKSHG ALSDSKSVSN
LSEARAQIAE PSTSRYFPSS CLDLNSPTSP TPTRHSDTRT LLSPSGRNNR
NEGTLDSRRT TTRHSKTMEE LKLPEHMDSS HSHSLSAPHE SFSYGLGYTS
PFSSQQRPHR HSMYVTRDKV RAKGLDGSLS IGQGMAARAN SLQLLSPQPG
EQLPPEMTVA RSSVKETSRE GTSSFHTRQK SEGGVYHDPH SDDGTAPKEN
RHLYNDPVPR RVGSFYRVPS PRPDNSFHEN NVSTRVSSLP SESSSGTNHS
KRQPAFDPWK SPENISHSEQ LKEKEKQGFF RSMKKKKKKS QTVPNSDSPD
LLTLQKSIHS ASTPSSRPKE WRPEKISDLQ TQSQPLKSLR KLLHLSSASN
HPASSDPRFQ PLTAQQTKNS FSEIRIHPLS QASGGSSNIR QEPAPKGRPA
LQLPDGGCDG RRQRHHSGPQ DRRFMLRTTE QQGEYFCCGD PKKPHTPCVP

NRALHRPISS PAPYPVLQVR GTSMCPTLQV RGTDAFSCPT QQSGFSFFVR HVMREALIHR AQVNQAALLT YHENAALTGK

Wildtype CDKL5 protein (Accession No. NP_001310218) (isoform 2 – human)

MKIPNIGNVM NKFEILGVVG EGAYGVVLKC RHKETHEIVA IKKFKDSEEN EEVKETTLRE LKMLRTLKQE NIVELKEAFR RRGKLYLVFE YVEKNMLELL EEMPNGVPPE KVKSYIYQLI KAIHWCHKND IVHRDIKPEN LLISHNDVLK LCDFGFARNL SEGNNANYTE YVATRWYRSP ELLLGAPYGK SVDMWSVGCI LGELSDGQPL FPGESEIDQL FTIQKVLGPL PSEQMKLFYS NPRFHGLRFP AVNHPQSLER RYLGILNSVL LDLMKNLLKL DPADRYLTEQ CLNHPTFQTQ RLLDRSPSRS AKRKPYHVES STLSNRNQAG KSTALQSHHR SNSKDIQNLS VGLPRADEGL PANESFLNGN LAGASLSPLH TKTYQASSQP GSTSKDLTNN NIPHLLSPKE AKSKTEFDFN IDPKPSEGPG TKYLKSNSRS QQNRHSFMES SQSKAGTLQP NEKQSRHSYI DTIPQSSRSP SYRTKAKSHG ALSDSKSVSN LSEARAQIAE PSTSRYFPSS CLDLNSPTSP TPTRHSDTRT LLSPSGRNNR NEGTLDSRRT TTRHSKTMEE LKLPEHMDSS HSHSLSAPHE SFSYGLGYTS PFSSQQRPHR HSMYVTRDKV RAKGLDGSLS IGQGMAARAN SLQLLSPQPG EQLPPEMTVARSSVKETSRE GTSSFHTRQK SEGGVYHDPH SDDGTAPKEN RHLYNDPVPR RVGSFYRVPS PRPDNSFHEN NVSTRVSSLP SESSSGTNHS KRQPAFDPWK SPENISHSEQ LKEKEKQGFF RSMKKKKKKS QTVPNSDSPD LLTLQKSIHS ASTPSSRPKE WRPEKISDLQ TQSQPLKSLR KLLHLSSASN HPASSDPRFQ PLTAQQTKNS FSEIRIHPLS QASGGSSNIR QEPAPKGRPA LQLPGQMDPG WHVSSVTRSA TEGPSYSEQL GAKSGPNGHP YNRTNRSRMP NLNDLKETAL

EXAMPLE 24. USE OF PRIME EDITING TO TARGET PATHOGENIC AOL1 ALLELES AS TREATMENT FOR NON-DIABETIC CHRONIC KIDNEY DISEASE

[1578] This Example designed PEgRNAs that are capable of targeting pathogenic APOL1 alleles for use with prime editing to treat or reduce the likelihood of developing a renal disease.

[1579] End-stage kidney failure (ESKD) is a growing problem that now affects over half a million individuals in the United States. The cost of caring for patients with ESKD is

currently over 40 billion dollars per year. In the U.S., the likelihood that subjects of African descent will develop ESKD is 4 to 5 times higher than for Americans without African ancestry. These facts are reflected in the disparity between the 12-13% of the U.S. population with African descent and the 40% of U.S. dialysis patients who are African-American. The epidemic of renal disease risk factors, such as obesity and metabolic syndrome, suggests that the magnitude of this problem will only increase.

[1580] There are no specific therapies for the vast majority of progressive kidney diseases. Some types of chronic renal disease progression can be slowed by blood pressure control with specific agents, but nephrologists cannot accurately predict which patients will respond. Moreover, while successful treatment typically slows progression, it neither prevents disease nor halts disease progression.

[1581] Recently it was determined that that specific genetic variants that alter the protein sequence of APOlipoprotein-L1 (APOL1) are associated with progressive kidney disease. Surprisingly, APOL1 kidney disease variants have a major impact on multiple different types of kidney disease including hypertension-associated end-stage renal disease (H-ESRD), focal segmental glomerulosclerosis (FSGS), and HIV-associated nephropathy (HIVAN). Individuals with these variant APOL1 alleles have a 7-30 fold increased risk for kidney disease. Based on the high frequency of these APOL1 risk alleles, more than 3.5 million African Americans likely have the high risk APOL1 genotype. African Americans without the high risk genotype have little excess risk compared with Americans of European ancestry.

[1582] Despite evidence that variants in the APOL1 gene cause renal disease, very little is known about the biology of its product, APOL1 , or its role in the kidney. APOL1 has a defined role in resistance to trypanosomes, and the G1 and G2 variants appear to have become common in Africa because they confer protection against the forms of trypanosomes that cause African Sleeping Sickness.

[1583] There still exists a need for therapies for kidney diseases in patients with one or more APOL1 risk alleles, which cause great morbidity and mortality with high economic impact in this and other subject populations.

[1584] This Example provides three exemplary PEgRNA design options based on a specific exemplary target sequence that may be used with prime editing to correct APOL1 defective alleles.

[1585] PEgRNA 1

[1586] Designing PEgRNAs for APOL1 allele rs73885319 (p.S342G). This represents a G→A correction in affected individuals. The target sequence is 5-GGAGTC**AAGCTCACGGATGTGGCCCC**TGTA(G-to-A)GCTTCTTTCTTGTG CTGGATGTAGTCTACCT-3.

[1587] The protospacer (bolded above) is AAGCTCACGGATGTGGCCCC. The selected PE comprises a SaCas9(D10A).

[1588] The primer binding sites can be:

[1589] GTGGCCCC

[1590] TGTGGCCCC

[1591] ATGTGGCCCC

[1592] GATGTGGCCCC

[1593] GGATGTGGCCCC

[1594] CGGATGTGGCCCC

[1595] ACGGATGTGGCCCC

[1596] CACGGATGTGGCCCC

[1597] TCACGGATGTGGCCCC

[1598] CTCACGGATGTGGCCCC.

[1599] The RT templates can be:

[1600] AAGAAGCTTACA

[1601] AAAGAAGCTTACA

[1602] GAAAGAAGCTTACA

[1603] AGAAAGAAGCTTACA

[1604] AAGAAAGAAGCTTACA

[1605] CAAGAAAGAAGCTTACA

[1606] ACAAGAAAGAAGCTTACA

[1607] CACAAGAAAGAAGCTTACA

[1608] GCACAAGAAAGAAGCTTACA

[1609] AGCACAAGAAAGAAGCTTACA

[1610] CAGCACAAGAAAGAAGCTTACA

[1611] CCAGCACAAGAAAGAAGCTTACA

[1612] TCCAGCACAAGAAAGAAGCTTACA

[1613] ATCCAGCACAAGAAAGAAGCTTACA.

[1614] The nicking template can be GCTTTGATTCGTACACGAGG.

[1615] PEgRNA 2

[1616] Designing PEgRNAs for APOL1 allele rs60910145. This represents a G→T correction in affected individuals.

[1617] The protospacer is GCTGGAGGAGAAGCTAAACA. The selected PE comprises SpCas9(D10A)-NG.

[1618] The primer binding sites can be:

[1619] GAAGCTAA

[1620] AGAAGCTAA

[1621] GAGAAGCTAA

[1622] GGAGAAGCTAA

[1623] AGGAGAAGCTAA

[1624] GAGGAGAAGCTAA

[1625] GGAGGAGAAGCTAA

[1626] TGGAGGAGAAGCTAA

[1627] CTGGAGGAGAAGCTAA

[1628] GCTGGAGGAGAAGCTAA.

[1629] The RT template can be (cannot end in C):

[1630] AGAATGT

[1631] GAGAATGT

[1632] TGAGAATGT

[1633] TTGAGAATGT

[1634] GTTGAGAATGT

[1635] TGTTGAGAATGT

[1636] TTGTTGAGAATGT

[1637] ATTGTTGAGAATGT

[1638] TATTGTTGAGAATGT

[1639] TTATTGTTGAGAATGT

[1640] ATTATTGTTGAGAATGT

[1641] AATTATTGTTGAGAATGT

[1642] TAATTATTGTTGAGAATGT

[1643] ATAATTATTGTTGAGAATGT.

[1644] The nicking templates can be:

[1645] CCTGTGGTCACAGTTCTTGG

[1646] CCACAGGGCAGGGCAGCCAC.

[1647] PEgRNA 3

[1648] Designing PEgRNAs for APOL1 allele rs71785313. This represents an insert, as follows: ATTCTCAACAA[insert: TAATTA]TAAGATTC.

[1649] The protospacer can be: TCTCAACAATAAGATTCTGC

[1650] The PE comprises SaKKH-PE2.

[1651] The primer binding site can be:

[1652] TTCTCAAC

[1653] ATTCTCAAC

[1654] CATTCTCAAC

[1655] ACATTCTCAAC

[1656] AACATTCTCAAC

[1657] AAACATTCTCAAC

[1658] TAAACATTCTCAAC

[1659] CTAAACATTCTCAAC

[1660] GCTAAACATTCTCAAC

[1661] AGCTAAACATTCTCAAC.

[1662] The RT template can be:

[1663] AATCTTATAATTATT

[1664] GAATCTTATAATTATT

[1665] AGAATCTTATAATTATT

[1666] CAGAATCTTATAATTATT

[1667] GCAGAATCTTATAATTATT

[1668] TGCAGAATCTTATAATTATT

[1669] CTGCAGAATCTTATAATTATT

[1670] CCTGCAGAATCTTATAATTATT

[1671] GCCTGCAGAATCTTATAATTATT

[1672] CGCCTGCAGAATCTTATAATTATT

[1673] CCGCCTGCAGAATCTTATAATTATT

[1674] TCCGCCTGCAGAATCTTATAATTATT

[1675] GTCCGCCTGCAGAATCTTATAATTATT

[1676] GGTCCGCCTGCAGAATCTTATAATTATT.

[1677] The nicking templates can be:

[1678] CCTGTGGTCACAGTTCTTGG

[1679] CCACAGGGCAGGGCAGCCAC.

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EMBODIMENTS

[1680] The following embodiments are within the scope of the present disclosure. Furthermore, the disclosure encompasses all variations, combinations, and permutations of these embodiments in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed embodiments is introduced into another listed embodiment in this section. For example, any listed embodiment that is dependent on another embodiment can be modified to include one or more limitations found in any other listed embodiment in this section that is dependent on the same base embodiment. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should it be understood that, in general, where the disclosure, or aspects of the disclosure, is/are referred to as comprising particular elements and/or features, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements and/or features. It is also noted that the terms "comprising" and "containing" are intended to be open and permits the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub–range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

GROUP 1. EMBODIMENTS 1-212

1. A fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase.

2. The fusion protein of embodiment 1, wherein the fusion protein is capable of carrying out genome editing by target-primed reverse transcription in the presence of an extended guide RNA.

3. The fusion protein of embodiment 1, wherein the napDNAbp has a nickase activity.

4. The fusion protein of embodiment 1, wherein the napDNAbp is a Cas9 protein or variant thereof.

5. The fusion protein of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

6. The fusion protein of embodiment 1, wherein the napDNAbp is Cas9 nickase (nCas9).

7. The fusion protein of embodiment 1, wherein the napDNAbp is selected from the group consisting of: Cas9, CasX, CasY, Cpf1, C2c1, C2c2, C2C3, and Argonaute and optionally has a nickase activity.

8. The fusion protein of embodiment 1, wherein the fusion protein when complexed with an extended guide RNA is capable of binding to a target DNA sequence.

9. The fusion protein of embodiment 8, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

10. The fusion protein of embodiment 8, wherein the binding of the fusion protein complexed to the extended guide RNA forms an R-loop.

11. The fusion protein of embodiment 10, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the extended guide RNA and the target strand, and (ii) the complementary non-target strand.

12. The fusion protein of embodiment 11, wherein the complementary non-target strand is nicked to form a reverse transcriptase priming sequence having a free 3´ end.

13. The fusion protein of embodiment 2, wherein the extended guide RNA comprises (a) a guide RNA, and (b) an RNA extension at the 5′ or the 3′ end of the guide RNA, or at an intramolecular location in the guide RNA.

14. The fusion protein of embodiment 13, wherein the RNA extension comprises (i) a reverse transcription template sequence comprising a desired nucleotide change, (ii) a reverse transcription primer binding site, and (iii) optionally, a linker sequence.

15. The fusion protein of embodiment 14, wherein the reverse transcription template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

16. The fusion protein of embodiment 13, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

17. The fusion protein of embodiment 15, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change.

18. The fusion protein of embodiment 15, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5′ end.

19. The fusion protein of embodiment 18, wherein the endogenous DNA sequence having the 5′ end is excised by the cell.

20. The fusion protein of embodiment 18, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

21. The fusion protein of embodiment 14, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence, or wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

22. The fusion protein of embodiment 1, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence to SEQ ID NO: 2.

23. The fusion protein of embodiment 1, wherein the napDNAbp comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 2-10.

24. The fusion protein of embodiment 1, wherein the reverse transcriptase comrises any one of the amino acid sequences of SEQ ID NO: 11-17.

25. The fusion protein of embodiment 1, wherein the reverse transcriptase comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 11-17.

26. The fusion protein of embodiment 1, wherein the reverse transcriptase is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

27. The fusion protein of any one of the previous embodiments, wherein the fusion protein comprises the structure NH_2-[napDNAbp]-[reverse transcriptase]-COOH; or NH_2-[reverse transcriptase]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

28. The fusion protein of embodiment 27, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 37-47.

29. The fusion protein of embodiment 14, wherein the desired nucleotide change is a single nucleotide change, an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

30. The fusion protein of embodiment 29, wherein the insert or deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 , at least 23 , at least 24 , at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50.

31. An extended guide RNA comprising a guide RNA and at least one RNA extension.

32. The extended guide RNA of embodiment 1, wherein the RNA extension is position at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

33. The extended guide RNA of embodiment 31, wherein the extended guide RNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

34. The extended guide RNA of embodiment 33, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

35. The extended guide RNA of embodiment 31, wherein the at least one RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

36. The extended guide RNA of embodiment 35, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

37. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

38. The extended guide RNA of embodiment 35, wherein the reverse transcription primer binding site sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

39. The extended guide RNA of embodiment 35, wherein the optional linker sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

40. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

41. The extended guide RNA of embodiment 40, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5′ end in the target DNA sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

42. The extended guide RNA of embodiment 41, wherein the endogenous single-stranded DNA having the free 5′ end is excised by the cell.

43. The extended guide RNA of embodiment 41, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

44. The extended guide RNA of embodiment 31, comprising the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

45. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

46. The extended guide RNA of embodiment 35, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

47. The extended guide RNA of embodiment 35, wherein the optional linker sequence is at least 1 nucleotide, or at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 nucleotides in length.

48. A complex comprising comprising a fusion protein of any one of embodiments 1-30 and an extended guide RNA.

49. The complex of embodiment 48, wherein the extended guide RNA comprises a guide RNA and an RNA extension at the 3´ or 5´ end of the guide RNA or at an intramolecular position in the guide RNA.

50. The complex of embodiment 48, wherein the extended guide RNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

51. The complex of embodiment 50, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

52. The complex of embodiment 49, wherein the at least one RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

53. The complex of embodiment 48, wherein the extended guide RNA comprises the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or

at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

54. The complex of embodiment 52, wherein the reverse transcription template sequence comprises a nucleotide sequence having at least 80%, or 85%, or 90%, or 95%, or 99% sequence identity with the endogenous DNA target.

55. The complex of embodiment 52, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

56. A complex comprising comprising a napDNAbp and an extended guide RNA.

57. The complex of embodiment 56, wherein the napDNAbp is a Cas9 nickase.

58. The complex of embodiment 56, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 2.

59. The complex of embodiment 57, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

60. The complex of embodiment 57, wherein the extended guide RNA comprises a guide RNA and an RNA extension at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

61. The complex of embodiment 57, wherein the extended guide RNA is capable of directing the napDNAbp to a target DNA sequence.

62. The complex of embodiment 61, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the spacer sequence hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

63. The complex of embodiment 61, wherein the RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

64. The complex of embodiment 57, wherein the extended guide RNA comprises the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

65. The complex of embodiment 63, wherein the reverse transcription template sequence comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

66. The complex of embodiment 63, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

67. The complex of embodiment 63, wherein the optional linker sequence is at least 1 nucleotide, or at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 nucleotides in length.

68. A polynucleotide encoding the fusion protein of any of embodiments 1-30.

69. A vector comprising the polynucleotide of embodiment 68.

70. A cell comprising the fusion protein of any of embodiments 1-30 and an extended guide RNA bound to the napDNAbp of the fusion protein.

71. A cell comprising a complex of any one of embodiments 48-67.

72. A pharmaceutical composition comprising: (i) a fusion protein of any of embodiments 1-30, the complex of embodiments 48-67, the polynucleotide of embodiment 68, or the vector of embodiment 69; and (ii) a pharmaceutically acceptable excipient.

73. A pharmaceutical composition comprising: (i) the complex of embodiments 48-67 (ii) reverse transcriptase provided in trans; and (iii) a pharmaceutically acceptable excipient.

74. A kit comprising a nucleic acid construct, comprising: (i) a nuleic acid sequencing encoding the fusion protein of any one of embodiments 1-30; and (ii) a promoter that drives expression of the sequence of (i).

75. A method for installing a desired nucleotide change in a double-stranded DNA sequence, the method comprising:

(i) contacting the double-stranded DNA sequence with a complex comprising a fusion protein and an extended guide RNA, wherein the fusion protein comprises a napDNAbp and a reverse transcriptase and wherein the extended guide RNA comprises a reverse transcription template sequence comprising the desired nucleotide change;

(ii) nicking the double-stranded DNA sequence on the non-target strand, thereby generating a free single-strand DNA having a 3′ end;

(iii) hybridizing the 3′ end of the free single-strand DNA to the reverse transcription template sequence, thereby priming the reverse transcriptase domain;

(iv) polymerizing a strand of DNA from the 3′ end, thereby generating a single-strand DNA flap comprising the desired nucleotide change;

(v) replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

76. The method of embodiment 75, wherein the step of (v) replacing comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

77. The method of embodiment 76, wherein the desired nucleotide change is a single nucleotide substitution, a deletion, or an insertion.

78. The method of embodiment 77, wherein the single nucleotide substitution is a transition or a transversion.

79. The method of embodiment 76, wherein the desired nucleotide change is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T

substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

80. The method of embodiment 76, wherein the desired nucleoid change converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

81. The method of embodiment 76, wherein the desired nucleotide change is an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

82. The method of embodiment 76, wherein the desired nucleotide change corrects a disease-associated gene.

83. The method of embodiment 82, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease.

84. The method of embodiment 82, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

85. The method of embodiment 76, wherein the napDNAbp is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9.

86. The method of embodiment 76, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2.

87. The method of embodiment 76, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

88. The method of embodiment 76, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 11-17.

89. The method of embodiment 76, wherein the reverse transcriptase domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 11-17.

90. The method of embodiment 76, wherein the extended guide RNA comprises an RNA extension at the 3` or 5` ends or at an intramolecular location in the guide RNA, wherein the RNA extension comprises the reverse transcription template sequence.

91. The method of embodiment 90, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

92. The method of embodiment 76, wherein the extended guide RNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 18-36.

93. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising:

(i) contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a guide RNA which targets the napDNAbp to the target locus, wherein the guide RNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change;

(ii) forming an exposed 3´ end in a DNA strand at the target locus;

(iii) hybridizing the exposed 3´ end to the RT template sequence to prime reverse transcription;

(iv) synthesizing a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence by reverse transcriptase;

(v) and incorporating the at least one desired nucleotide change into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

94. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a transition.

95. The method of embodiment 94, wherein the transition is selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

96. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a transversion.

97. The method of embodiment 96, wherein the transversion is selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

98. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

99. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

100. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a correction to a disease-associated gene.

101. The method of embodiment 100, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease.

102. The method of embodiment 100, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

103. The method of embodiment 93, wherein the napDNAbp is a nuclease active Cas9 or variant thereof.

104. The method of embodiment 93, wherein the napDNAbp is a nuclease inactive Cas9 (dCas9) or Cas9 nickase (nCas9), or a variant thereof.

105. The method of embodiment 93, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2.

106. The method of embodiment 93, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

107. The method of embodiment 93, wherein the reverse transcriptase is introduced in trans.

108. The method of embodiment 93, wherein the napDNAbp comprises a fusion to a reverse transcriptase.

109. The method of embodiment 93, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 11-17.

110. The method of embodiment 93, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 11-17.

111. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises nicking the DNA strand with a nuclease.

112. The method of embodiment 111, wherein the nuclease is the napDNAbp, is provided as a fusion domain of napDNAbp, or is provided in trans.

113. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises contacting the DNA strand with a chemical agent.

114. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises introducing a replication error.

115. The method of embodiment 93, wherein the step of contacting the DNA molecule with the napDNAbp and the guide RNA forms an R-loop.

116. The method of embodiment 115, wherein the DNA strand in which the exposed 3' end is formed is in the R-loop.

117. The method of embodiment 93, wherein guide RNA comprises an extended portion that comprises the reverse transcriptase (RT) template sequence.

118. The method of embodiment 117, wherein the extended portion is at the 3´ end of the guide RNA, the 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

119. The method of embodiment 93, wherein the guide RNA further comprises a primer binding site.

120. The method of embodiment 93, wherein the guide RNA further comprises a spacer sequence.

121. The method of embodiment 93, wherein the RT template sequence is homologous to the corresponding endogenous DNA.

122. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase

and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change; and (c) incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

123. The method of embodiment 122, wherein the RT template is located at the 3´ end of the guide RNA, the 5′ end of the guide RNA, or at an intramolecular location in the guide RNA.

124. The method of embodiment 122, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

125. The method of clami 122, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

126. The method of clami 122, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

127. The method of embodiment 122, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

128. A polynucleotide encoding the extended guide RNA of any one of embodiments 31-47.

129. A vector comprising the polynucleotide of embodiment 128.

130. A cell comprising the vector of embodiment 129.

131. The fusion protein of any of embodiments 1-30, wherein the reverse transcriptase is an error-prone reverse transcriptase.

132. A method for mutagenizing a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a mutagenized single strand DNA; and (c) incorporating the mutagenized single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

133. The method of embodiment 132, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

134. The method of embodiment 132, wherein the napDNAbp is a Cas9 nickase (nCas9).

135. The method of embodiment 132, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

136. The method of embodiment 132, wherein the guide RNA comprises SEQ ID NOs: 222.

137. The method of embodiment 132, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3' end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

138. A method for replacing a trinucleotide repeat expansion mutation in a target DNA molecule with a healthy sequence comprising a healthy number of repeat trinucleotides, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising the replacement sequence, wherein said fusion protein intr; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the replacement

sequence; and (c) incorporating the single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

139. The method of embodiment 138, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

140. The method of embodiment 138, wherein the napDNAbp is a Cas9 nickase (nCas9).

141. The method of embodiment 138, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

142. The method of embodiment 138, wherein the guide RNA comprises SEQ ID NOs: 222.

143. The method of embodiment 138, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3' end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

144. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation is associated with Huntington's Disease, Fragile X syndrome, or Friedreich's ataxia.

145. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of CAG triplets.

146. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of GAA triplets.

147. A method of installing a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the functional moiety; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target

nucleotide sequence that encodes a fusion protein comprising the protein of interest and the functional moiety.

148. The method of embodiment 147, wherein functional moiety is peptide tag.

149. The method of embodiment 148, wherein the peptide tag is an affinity tag, solubilization tag, chromatography tag, epitope tag, or a fluorescence tag.

150. The method of embodiment 148, wherein the peptide tag is selected from the group consisting of: AviTag (SEQ ID NO: 245); C-tag (SEQ ID NO: 246); Calmodulin-tag (SEQ ID NO: 247); polyglutamate tag (SEQ ID NO: 248); E-tag (SEQ ID NO: 249); FLAG-tag (SEQ ID NO: 250); HA-tag (SEQ ID NO: 251); His-tag (SEQ ID NOs: 252-262); Myc-tag (SEQ ID NO: 263); NE-tag (SEQ ID NO: 264); Rho1D4-tag (SEQ ID NO: 265); S-tag (SEQ ID NO: 266); SBP-tag (SEQ ID NO: 267); Softag-1 (SEQ ID NO: 268); Softag-2 (SEQ ID NO: 269); Spot-tag (SEQ ID NO: 270); Strep-tag (SEQ ID NO: 271); TC tag (SEQ ID NO: 272); Ty tag (SEQ ID NO: 273); V5 tag (SEQ ID NO: 274); VSV-tag (SEQ ID NO: 275); and Xpress tag (SEQ ID NO: 276).

151. The method of embodiment 148, wherein the peptide tag is selected from the group consisting of: AU1 epitope (SEQ ID NO: 278); AU5 epitope (SEQ ID NO: 279); Bacteriophage T7 epitope (T7-tag) (SEQ ID NO: 280); Bluetongue virus tag (B-tag) (SEQ ID NO: 281); E2 epitope (SEQ ID NO: 282); Histidine affinity tag (HAT) (SEQ ID NO: 283); HSV epitope (SEQ ID NO: 284); Polyarginine (Arg-tag) (SEQ ID NO: 285); Polyaspartate (Asp-tag) (SEQ ID NO: 286); Polyphenylalanine (Phe-tag) (SEQ ID NO: 287); S1-tag (SEQ ID NO: 288); S-tag (SEQ ID NO: 289); and VSV-G (SEQ ID NO: 290).

152. The method of embodiment 147, wherein the functional moiety is an immunoepitope.

153. The method of embodiment 152, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 398); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin

epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

154. The method of embodiment 147, wherein the functional moiety alters the localization of the protein of interest.

155. The method of embodiment 147, wherein the functional moiety is a degradation tag such that the degradation rate of the protein of interest is altered.

156. The method of embodiment 155, wherein the degradation tag.

157. The method of embodiment 147, wherein the functional moiety is a small molecule binding domain.

158. The method of embodiment 157, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

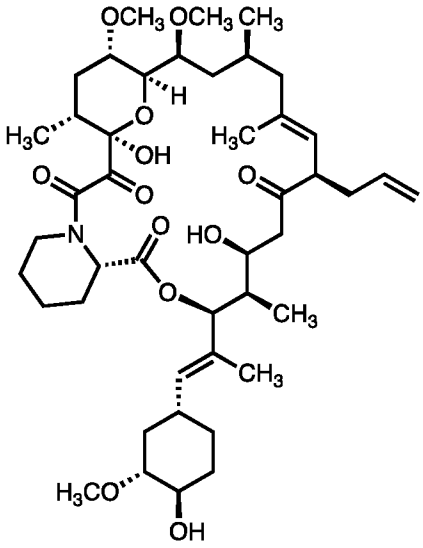
159. The method of embodiment 157, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

160. The method of embodiment 157, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 492-494.

161. The method of embodiment 157, wherein the small molecule binding domain is installed in two or more proteins of interest.

162. The method of embodiment 161, wherein the two or more proteins of interest may dimerize upon contacting with a small molecule.

163. The method of embodiment 157, wherein the small molecule is a dimer of a small molecule selected from the group consisting of:



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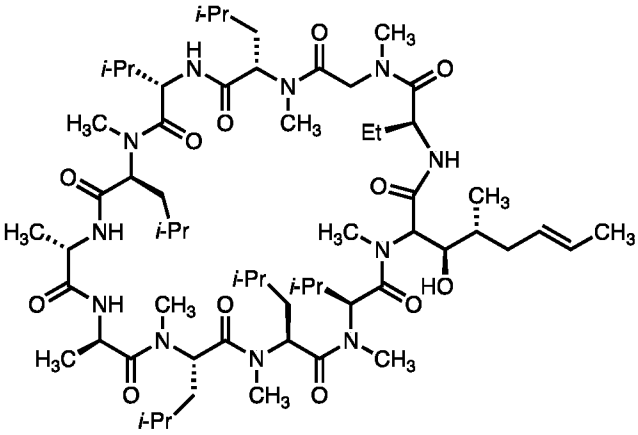

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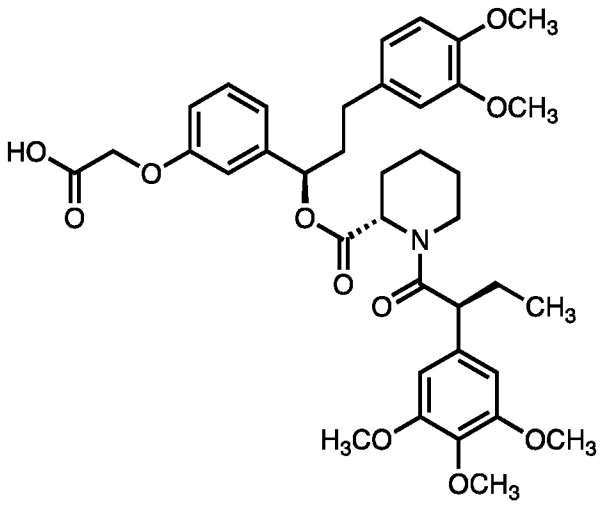

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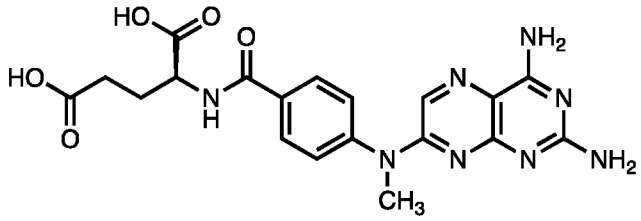

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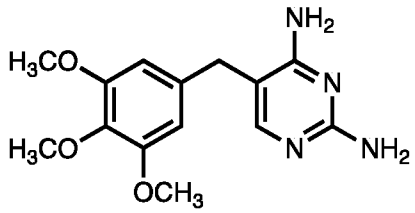

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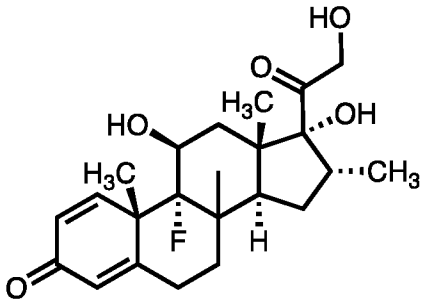

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164. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair

and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

165. The method of embodiment 164, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 398); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

166. The method of embodiment 164, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

167. The method of embodiment 164, wherein the napDNAbp is a Cas9 nickase (nCas9).

168. The method of embodiment 164, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

169. The method of embodiment 164, wherein the guide RNA comprises SEQ ID NOs: 222.

170. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein

the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the small molecule dimerization domain.

171. The method of embodiment 170, further comprising conducting the method on a second protein of interest.

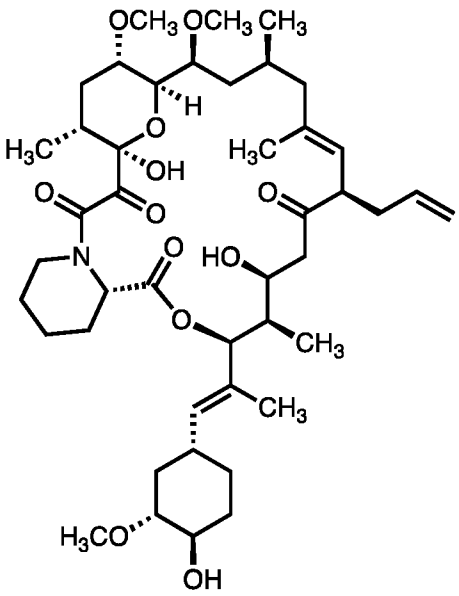
172. The method of embodiment 171, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

173. The method of embodiment 170, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

174. The method of embodiment 170, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

175. The method of embodiment 170, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 492-494.

176. The method of embodiment 170, wherein the small molecule is a dimer of a small molecule selected from the group consisting of:



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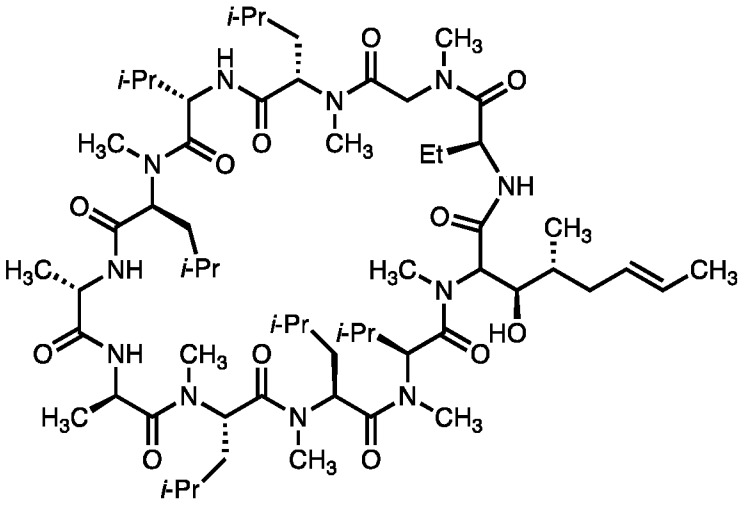

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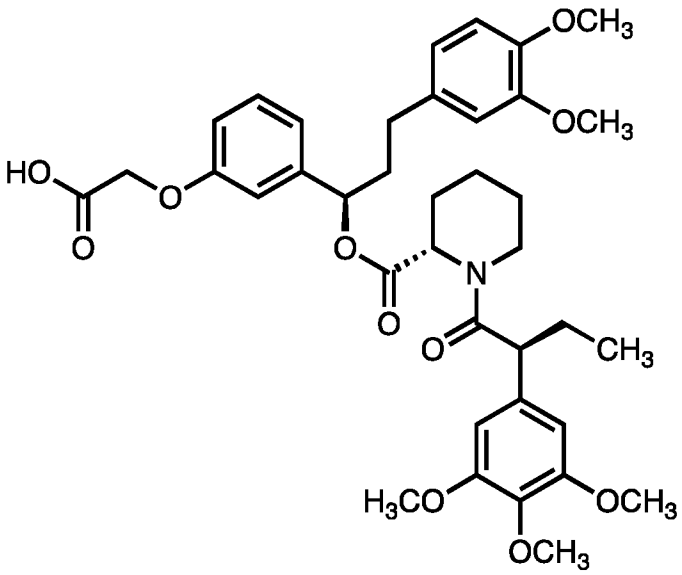

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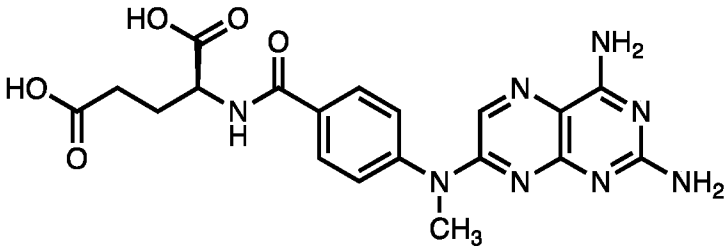

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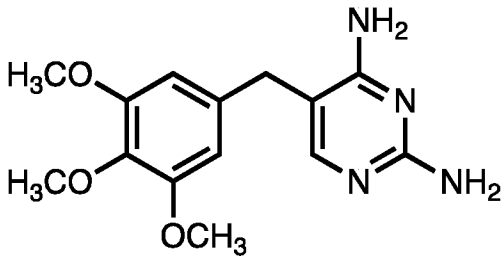

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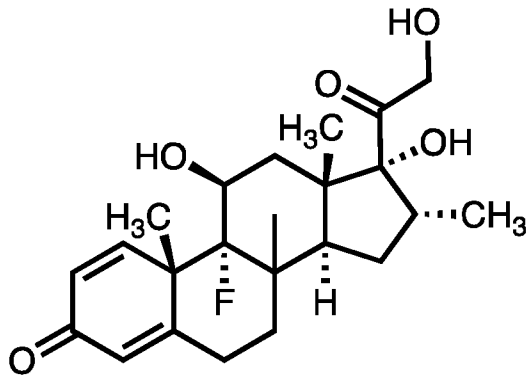

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177. The method of embodiment 170, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

178. The method of embodiment 170, wherein the napDNAbp is a Cas9 nickase (nCas9).

179. The method of embodiment 170, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

180. The method of embodiment 170, wherein the guide RNA comprises SEQ ID NOs: 222.

181. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

182. The method of embodiment 181, wherein the peptide tag is used for purification and/or detection of the protein.

183. The method of embodiment 181, wherein the peptide tag is a poly-histidine (e.g., HHHHHH), FLAG (e.g., DYKDDDDK), V5 (e.g., GKPIPNPLLGLDST), GCN4, HA (e.g., YPYDVPDYA), Myc (e.g. EQKLISEED), GST….etc.

184. The method of embodiment 181, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 245-290.

185. The method of embodiment 181, wherein the peptide tag is fused to the protein by a linker.

186. The method of embodiment 181, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

187. The method of embodiment 181, wherein the linker has an amino acid sequence of SEQ ID NO: 127, 165-176, 446, 453, and 767- 769.

188. The method of embodiment 181, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 18-25.

189. The method of embodiment 181, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

190. The method of embodiment 181, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

191. A method of preventing or halting the progression of a prion disease by installing on or more protective mutations into PRNP encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the protective mutation; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a PRNP comprising a protective mutation and which is resistant to misfolding.

192. The method of embodiment 191, wherein the prion disease is a human prion disease.

193. The method of embodiment 191, wherein the prion disease is an animal prion disease.

194. The method of embodiment 192, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, or Kuru.

195. The method of embodiment 193, wherein the prion disease is Bovine Spongiform Encephalopathy (BSE or “mad cow disease”), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy.

196. The method of embodiment 191, wherein the wildtype PRNP amino acid sequence is SEQ ID NOs: 291-292.

197. The method of embodiment 191, wherein the method results in a modified PRNP amino acid sequence selected from the group consisting of SEQ ID NOs: 293-323, wherein said modified PRNP protein is resistant to misfolding.

198. The method of embodiment 191, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

199. The method of embodiment 191, wherein the napDNAbp is a Cas9 nickase (nCas9).

200. The method of embodiment 191, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

201. The method of embodiment 191, wherein the guide RNA comprises SEQ ID NOs: 222.

202. A method of installing a ribonucleotide motif or tag in an RNA of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the ribonucleotide motif or tag; (b) polymerizing a single strand DNA sequence encoding the ribonucleotide motif or tag; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified RNA of interest comprising the ribonucleotide motif or tag.

203. The method of embodiment 202, wherein ribonucleotide motif or tag is a detection moiety.

204. The method of embodiment 202, wherein the ribonucleotide motif or tag affects the expression level of the RNA of interest.

205. The method of embodiment 202, wherein the ribonucleotide motif or tag affects the transport or subcellular location of the RNA of interest.

206. The method of embodiment 202, wherein the ribonucleotide motif or tag is selected from the group consisting of SV40 type 1, SV40 type 2, SV40 type 3, hGH, BGH, rbGlob,

TK, MALAT1 ENE-mascRNA, KSHV PAN ENE, Smbox/U1 snRNA box, U1 snRNA 3' box, tRNA-lysine, broccoli aptamer, spinach aptamer, mango aptamer, HDV ribozyme, and m6A.

207. The method of embodiment 202, wherein the PEgRNA comprises SEQ ID NOs: 18-25.

208. The method of embodiment 202, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

209. The method of embodiment 202, wherein the napDNAbp is a Cas9 nickase (nCas9).

210. The method of embodiment 202, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

211. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

212. The method of embodiment 211, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, SUMOylation state of the protein of interest.

GROUP 2. EMBODIMENTS 213-424

213. A fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase.

214. The fusion protein of embodiment 213, wherein the fusion protein is capable of carrying out prime editing in the presence of an prime editing guide RNA (PEgRNA).

215. The fusion protein of embodiment 213, wherein the napDNAbp has a nickase activity.

216. The fusion protein of embodiment 213, wherein the napDNAbp is a Cas9 protein or variant thereof.

217. The fusion protein of embodiment 213, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

218. The fusion protein of embodiment 213, wherein the napDNAbp is Cas9 nickase (nCas9).

219. The fusion protein of embodiment 213, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

220. The fusion protein of embodiment 213, wherein the fusion protein when complexed with a PEgRNA is capable of binding to a target DNA sequence.

221. The fusion protein of embodiment 220, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

222. The fusion protein of embodiment 220, wherein the binding of the fusion protein complexed to the PEgRNA forms an R-loop.

223. The fusion protein of embodiment 222, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the PEgRNA and the target strand, and (ii) the complementary non-target strand.

224. The fusion protein of embodiment 223, wherein the complementary non-target strand is nicked to form a priming sequence having a free 3´ end.

225. The fusion protein of embodiment 214, wherein the PEgRNA comprises (a) a guide RNA and (b) an extension arm at the 5´ or the 3´ end of the guide RNA, or at an intramolecular location in the guide RNA.

226. The fusion protein of embodiment 225, wherein the extension arm comprises (i) a DNA synthesis template sequence comprising a desired nucleotide change, and (ii) a primer binding site.

227. The fusion protein of embodiment 226, wherein the DNA synthesis template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

228. The fusion protein of embodiment 225, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

229. The fusion protein of embodiment 227, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change.

230. The fusion protein of embodiment 227, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5' end.

231. The fusion protein of embodiment 230, wherein the endogenous DNA sequence having the 5´ end is excised by the cell.

232. The fusion protein of embodiment 230, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

233. The fusion protein of embodiment 226, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence, or wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,

19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

234. The fusion protein of embodiment 213, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence to SEQ ID NO: 2.

235. The fusion protein of embodiment 213, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

236. The fusion protein of embodiment 213, wherein the polymerase is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 11-17.

237. The fusion protein of embodiment 213, wherein the polymerase is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 11-17.

238. The fusion protein of embodiment 213, wherein the polymerase is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

239. The fusion protein of any one of the previous embodiments, wherein the fusion protein comprises the structure NH_2-[napDNAbp]-[polymerase]-COOH; or NH_2-[polymerase]-[napDNAbp]- COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

240. The fusion protein of embodiment 239, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 37-47.

241. The fusion protein of embodiment 226, wherein the desired nucleotide change is a single nucleotide change, an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

242. The fusion protein of embodiment 241, wherein the insert or deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at

least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 , at least 23 , at least 24 , at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50.

243. A PEgRNA comprising a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template.

244. The PEgRNA of embodiment 241, wherein the nucleic acid extension arm is position at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA, and wherein the nucleic acid extension arm is DNA or RNA.

245. The PEgRNA of embodiment 242, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

246. The PEgRNA of embodiment 245, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

247. The PEgRNA of embodiment 243, wherein the at least one nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

248. The PEgRNA of embodiment 247, wherein the nucleic acid extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

249. The PEgRNA of embodiment 247, wherein the DNA synthesis template is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

250. The PEgRNA of embodiment 247, wherein the primer binding site is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

251. The PEgRNA of embodiment 243, further comprising at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA- protein recruitment domain.

252. The PEgRNA of embodiment 247, wherein the DNA synthesis template encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

253. The PEgRNA of embodiment 252, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5´ end in the target DNA sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

254. The PEgRNA of embodiment 253, wherein the endogenous single-stranded DNA having the free 5´ end is excised by the cell.

255. The PEgRNA of embodiment 253, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

256. The PEgRNA of embodiment 243, comprising the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

257. The PEgRNA of embodiment 247, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

258. The PEgRNA of embodiment 247, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

259. The PEgRNA of embodiment 251, wherein the at least one additional structure is located at the 3' or 5' end of the PEgRNA.

260. A complex comprising comprising a fusion protein of any one of embodiments 213-242 and an PEgRNA.

261. The complex of embodiment 260, wherein the PEgRNA comprises a guide RNA and an nucleic acid extension arm at the 3´ or 5´ end of the guide RNA or at an intramolecular position in the guide RNA.

262. The complex of embodiment 260, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

263. The complex of embodiment 262, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

264. The complex of embodiment 261, wherein the at least one nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

265. The complex of embodiment 260, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

266. The complex of embodiment 264, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

267. The complex of embodiment 264, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

268. A complex comprising comprising a napDNAbp and an PEgRNA.

269. The complex of embodiment 268, wherein the napDNAbp is a Cas9 nickase.

270. The complex of embodiment 268, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2.

271. The complex of embodiment 268, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

272. The complex of embodiment 268, wherein the PEgRNA comprises a guide RNA and a nucleic acid extension arm at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

273. The complex of embodiment 268, wherein the PEgRNA is capable of directing the napDNAbp to a target DNA sequence.

274. The complex of embodiment 272, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the spacer sequence of the PEgRNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

275. The complex of embodiment 273, wherein the nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

276. The complex of embodiment 269, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 18-36.

277. The complex of embodiment 276, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

278. The complex of embodiment 276, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

279. The complex of embodiment 276, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

280. A polynucleotide encoding the fusion protein of any of embodiments 213-242.

281. A vector comprising the polynucleotide of embodiment 280.

282. A cell comprising the fusion protein of any of embodiments 213-242 and an PEgRNA bound to the napDNAbp of the fusion protein.

283. A cell comprising a complex of any one of embodiments 260-279.

284. A pharmaceutical composition comprising: (i) a fusion protein of any of embodiments 213-242, the complex of embodiments 260-279, the polynucleotide of embodiment 68, or the vector of embodiment 69; and (ii) a pharmaceutically acceptable excipient.

285. A pharmaceutical composition comprising: (i) the complex of embodiments 260-279 (ii) a polymerase provided in trans; and (iii) a pharmaceutically acceptable excipient.

286. A kit comprising a nucleic acid construct, comprising: (i) a nuleic acid sequencing encoding the fusion protein of any one of embodiments 213-242; and (ii) a promoter that drives expression of the sequence of (i).

287. A method for installing a desired nucleotide change in a double-stranded DNA sequence, the method comprising:

(i) contacting the double-stranded DNA sequence with a complex comprising a fusion protein and a PEgRNA, wherein the fusion protein comprises a napDNAbp and a polymerase and wherein the PEgRNA comprises a DNA synthesis template comprising the desired nucleotide change and a primer binding site;

(ii) nicking the double-stranded DNA sequence, thereby generating a free single-strand DNA having a 3´ end;

(iii) hybridizing the 3` end of the free single-strand DNA to the primer binding site, thereby priming the polymerase;

(iv) polymerizing a strand of DNA from the 3´ end hybridized to the primer binding site, thereby generating a single-strand DNA flap comprising the desired nucleotide change and which is complementary to the DNA synthesis template;

(v) replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA

flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

288. The method of embodiment 287, wherein the step of (v) replacing comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

289. The method of embodiment 288, wherein the desired nucleotide change is a single nucleotide substitution, a deletion, or an insertion.

290. The method of embodiment 289, wherein the single nucleotide substitution is a transition or a transversion.

291. The method of embodiment 288, wherein the desired nucleotide change is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

292. The method of embodiment 288, wherein the desired nucleoid change converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

293. The method of embodiment 288, wherein the desired nucleotide change is an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

294. The method of embodiment 288, wherein the desired nucleotide change corrects a disease-associated gene.

295. The method of embodiment 294, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase

(ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

296. The method of embodiment 294, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

297. The method of embodiment 287, wherein the napDNAbp is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9.

298. The method of embodiment 287, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 2.

299. The method of embodiment 287, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

300. The method of embodiment 287, wherein the polymerase is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 11-17.

301. The method of embodiment 287, wherein the polymerase is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 11-17.

302. The method of embodiment 287, wherein the PEgRNA comprises a nucleic acid extension arm at the 3` or 5` ends or at an intramolecular location in the guide RNA, wherein the extension arm comprises the DNA synthesis template sequence and the primer binding site.

303. The method of embodiment 302, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at

least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

304. The method of embodiment 287, wherein the PEgRNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 18-36.

305. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising:

(i) contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a PEgRNA which targets the napDNAbp to the target locus, wherein the PEgRNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change and a primer binding site;

(ii) forming an exposed 3´ end in a DNA strand at the target locus;

(iii) hybridizing the exposed 3´ end to the primer binding site to prime reverse transcription;

(iv) synthesizing a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence by reverse transcriptase;

(v) and incorporating the at least one desired nucleotide change into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

306. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a transition.

307. The method of embodiment 306, wherein the transition is selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

308. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a transversion.

309. The method of embodiment 308, wherein the transversion is selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

310. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (9) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

311. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

312. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a correction to a disease-associated gene.

313. The method of embodiment 312, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

314. The method of embodiment 312, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

315. The method of embodiment 305, wherein the napDNAbp is a nuclease active Cas9 or variant thereof.

316. The method of embodiment 305, wherein the napDNAbp is a nuclease inactive Cas9 (dCas9) or Cas9 nickase (nCas9), or a variant thereof.

317. The method of embodiment 305, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 2.

318. The method of embodiment 305, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 2-10.

319. The method of embodiment 305, wherein the reverse transcriptase is introduced *in trans*.

320. The method of embodiment 305, wherein the napDNAbp comprises a fusion to a reverse transcriptase.

321. The method of embodiment 305, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 11-17.

322. The method of embodiment 305, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 11-17.

323. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises nicking the DNA strand with a nuclease.

324. The method of embodiment 323, wherein the nuclease is provided is provided *in trans*.

325. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises contacting the DNA strand with a chemical agent.

326. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises introducing a replication error.

327. The method of embodiment 305, wherein the step of contacting the DNA molecule with the napDNAbp and the guide RNA forms an R-loop.

328. The method of embodiment 327, wherein the DNA strand in which the exposed 3' end is formed is in the R-loop.

329. The method of embodiment 315, wherein the PEgRNA comprises an extension arm that comprises the reverse transcriptase (RT) template sequence and the primer binding site.

330. The method of embodiment 329, wherein the extension arm is at the 3´ end of the guide RNA, the 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

331. The method of embodiment 305, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

332. The method of embodiment 305, wherein the PEgRNA further comprises a homology arm.

333. The method of embodiment 305, wherein the RT template sequence is homologous to the corresponding endogenous DNA.

334. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change; and (c) incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

335. The method of embodiment 334, wherein the RT template is located at the 3´ end of the guide RNA, the 5' end of the guide RNA, or at an intramolecular location in the guide RNA.

336. The method of embodiment 334, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

337. The method of embodiment 334, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

338. The method of clami 334, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

339. The method of embodiment 334, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

340. A polynucleotide encoding the PEgRNA of any one of embodiments 243-259.

341. A vector comprising the polynucleotide of embodiment 340.

342. A cell comprising the vector of embodiment 341.

343. The fusion protein of embodiment 213, wherein the polymerase is an error-prone reverse transcriptase.

344. A method for mutagenizing a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a mutagenized single strand DNA; and (c) incorporating the mutagenized single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

345. The method of any prior embodiment, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

346. The method of any prior embodiment, wherein the napDNAbp is a Cas9 nickase (nCas9).

347. The method of embodiment 344, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

348. The method of embodiment 344, wherein the guide RNA comprises SEQ ID NOs: 26-36.

349. The method of embodiment 344, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3' end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

350. A method for replacing a trinucleotide repeat expansion mutation in a target DNA molecule with a healthy sequence comprising a healthy number of repeat trinucleotides, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising DNA synthesis template comprising the replacement sequence and a primer binding site; (b) conducting prime editing to generate a single strand DNA comprising the replacement sequence; and (c) incorporating the single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

351. The method of embodiment 350, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

352. The method of embodiment 350, wherein the napDNAbp is a Cas9 nickase (nCas9).

353. The method of embodiment 350, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

354. The method of embodiment 350, wherein the guide RNA comprises SEQ ID NOs: 26-36.

355. The method of embodiment 350, wherein the step of (b) conducting prime editing comprises generating a 3' end primer binding sequence at the target locus that is capable of priming polymerase by annealing to the primer binding site on the guide RNA.

356. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation is associated with Huntington's Disease, Fragile X syndrome, or Friedreich's ataxia.

357. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of CAG triplets.

358. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of GAA triplets.

359. A method of installing a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising DNA synthesis template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the functional moiety; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the functional moiety.

360. The method of embodiment 359, wherein functional moiety is peptide tag.

361. The method of embodiment 360, wherein the peptide tag is an affinity tag, solubilization tag, chromatography tag, epitope or immunoepitope tag, or a fluorescence tag.

362. The method of embodiment 360, wherein the peptide tag is selected from the group consisting of: AviTag (SEQ ID NO: 245); C-tag (SEQ ID NO: 246); Calmodulin-tag (SEQ ID NO: 247); polyglutamate tag (SEQ ID NO: 248); E-tag (SEQ ID NO: 249); FLAG-tag (SEQ ID NO: 250); HA-tag (SEQ ID NO: 251); His-tag (SEQ ID NOs: 252-262); Myc-tag (SEQ ID NO: 263); NE-tag (SEQ ID NO: 264); Rho1D4-tag (SEQ ID NO: 265); S-tag (SEQ ID NO: 266); SBP-tag (SEQ ID NO: 267); Softag-1 (SEQ ID NO: 268); Softag-2 (SEQ ID NO: 269); Spot-tag (SEQ ID NO: 270); Strep-tag (SEQ ID NO: 271); TC tag (SEQ ID NO:

272); Ty tag (SEQ ID NO: 273); V5 tag (SEQ ID NO: 274); VSV-tag (SEQ ID NO: 275); and Xpress tag (SEQ ID NO: 276).

363. The method of embodiment 360, wherein the peptide tag is selected from the group consisting of: AU1 epitope (SEQ ID NO: 278); AU5 epitope (SEQ ID NO: 279); Bacteriophage T7 epitope (T7-tag) (SEQ ID NO: 280); Bluetongue virus tag (B-tag) (SEQ ID NO: 281); E2 epitope (SEQ ID NO: 282); Histidine affinity tag (HAT) (SEQ ID NO: 283); HSV epitope (SEQ ID NO: 284); Polyarginine (Arg-tag) (SEQ ID NO: 285); Polyaspartate (Asp-tag) (SEQ ID NO: 286); Polyphenylalanine (Phe-tag) (SEQ ID NO: 287); S1-tag (SEQ ID NO: 288); S-tag (SEQ ID NO: 289); and VSV-G (SEQ ID NO: 290).

364. The method of embodiment 359, wherein the functional moiety is an immunoepitope.

365. The method of embodiment 364, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 398); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

366. The method of embodiment 359, wherein the functional moiety alters the localization of the protein of interest.

367. The method of embodiment 359, wherein the functional moiety is a degradation tag such that the degradation rate of the protein of interest is altered.

368. The method of embodiment 367, wherein the degradation tag results in the elimination of the tagged protein.

369. The method of embodiment 359, wherein the functional moiety is a small molecule binding domain.

370. The method of embodiment 359, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

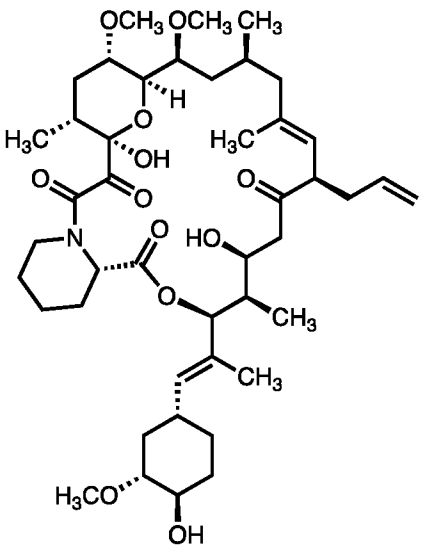
371. The method of embodiment 359, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

372. The method of embodiment 359, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 492-494.

373. The method of embodiment 359, wherein the small molecule binding domain is installed in two or more proteins of interest.

374. The method of embodiment 373, wherein the two or more proteins of interest may dimerize upon contacting with a small molecule.

375. The method of embodiment 369, wherein the small molecule is a dimer of a small molecule selected from the group consisting of:



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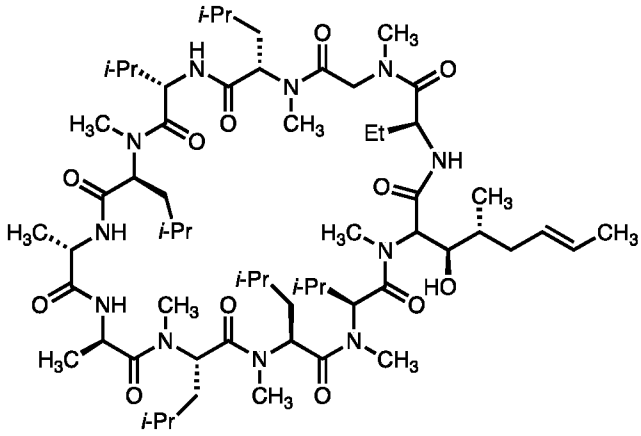

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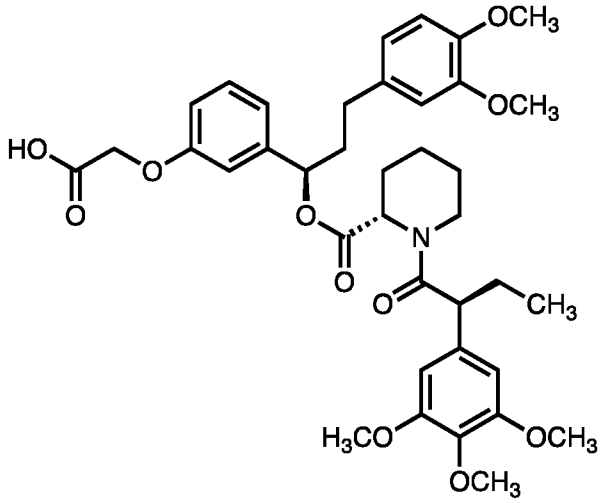

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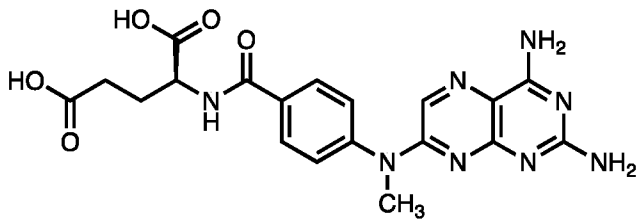

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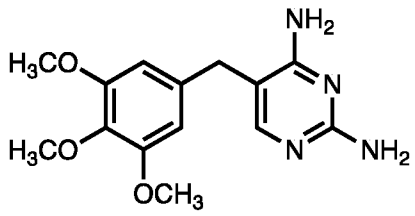

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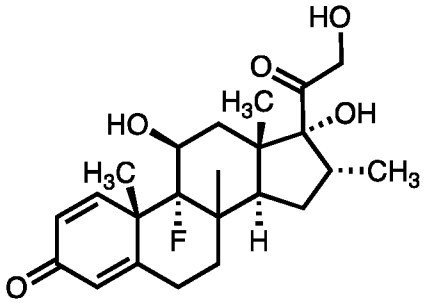

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376. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

377. The method of embodiment 376, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 398); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ

ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

378. The method of embodiment 376, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

379. The method of embodiment 376, wherein the napDNAbp is a Cas9 nickase (nCas9).

380. The method of embodiment 376, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

381. The method of embodiment 376, wherein the PEgRNA comprises SEQ ID NOs: 26-36.

382. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the small molecule dimerization domain.

383. The method of embodiment 382, further comprising conducting the method on a second protein of interest.

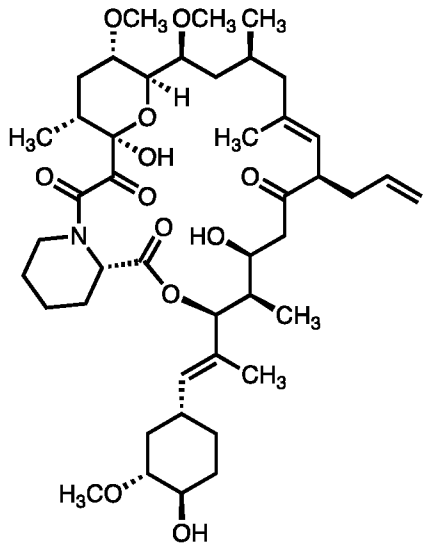
384. The method of embodiment 383, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

385. The method of embodiment 382, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

386. The method of embodiment 382, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

387. The method of embodiment 382, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 492-494.

388. The method of embodiment 382, wherein the small molecule is a dimer of a small molecule selected from the group consisting of:



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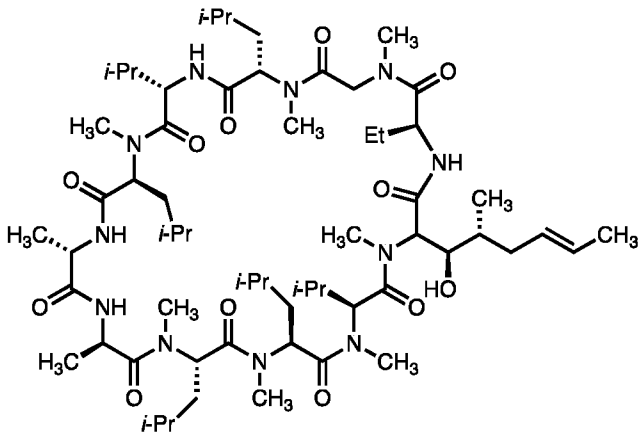

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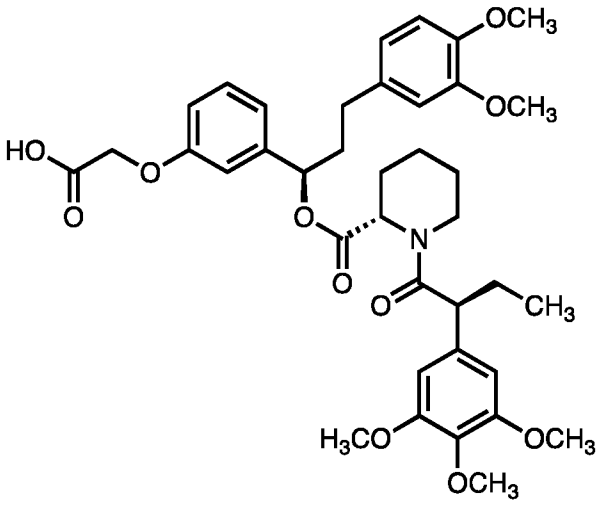

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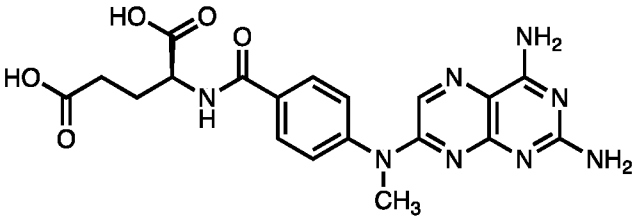

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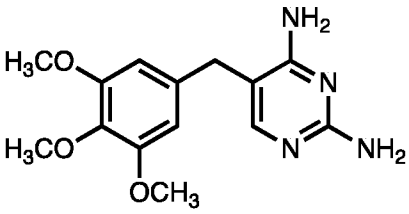

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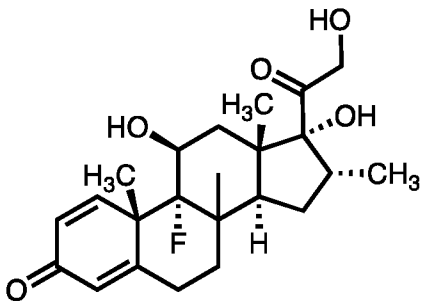


389. The method of embodiment 382, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

390. The method of embodiment 382, wherein the napDNAbp is a Cas9 nickase (nCas9).

391. The method of embodiment 382, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

392. The method of embodiment 382, wherein the PEgRNA comprises SEQ ID NOs: 26-36.

393. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

394. The method of embodiment 383, wherein the peptide tag is used for purification and/or detection of the protein.

395. The method of embodiment 383, wherein the peptide tag is a poly-histidine (e.g., HHHHHH), FLAG (e.g., DYKDDDDK), V5 (e.g., GKPIPNPLLGLDST), GCN4, HA (e.g., YPYDVPDYA), Myc (e.g. EQKLISEED), or GST.

396. The method of embodiment 383, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 245-290.

397. The method of embodiment 383, wherein the peptide tag is fused to the protein by a linker.

398. The method of embodiment 383, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

399. The method of embodiment 383, wherein the linker has an amino acid sequence of SEQ ID NO: 37-47.

400. The method of embodiment 383, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 18-25.

401. The method of embodiment 383, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

402. The method of embodiment 383, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

403. A method of preventing or halting the progression of a prion disease by installing on or more protective mutations into PRNP encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the protective mutation; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a PRNP comprising a protective mutation and which is resistant to misfolding.

404. The method of embodiment 403, wherein the prion disease is a human prion disease.

405. The method of embodiment 403, wherein the prion disease is an animal prion disease.

406. The method of embodiment 404, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, or Kuru.

407. The method of embodiment 403, wherein the prion disease is Bovine Spongiform Encephalopathy (BSE or "mad cow disease"), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy.

408. The method of embodiment 403, wherein the wildtype PRNP amino acid sequence is SEQ ID NOs: 291-292.

409. The method of embodiment 403, wherein the method results in a modified PRNP amino acid sequence selected from the group consisting of SEQ ID NOs: 293-323, wherein said modified PRNP protein is resistant to misfolding.

410. The method of embodiment 403, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

411. The method of embodiment 403, wherein the napDNAbp is a Cas9 nickase (nCas9).

412. The method of embodiment 403, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

413. The method of embodiment 403, wherein the PEgRNA comprises SEQ ID NOs: 26-36.

414. A method of installing a ribonucleotide motif or tag in an RNA of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the ribonucleotide motif or tag; (b) polymerizing a single strand DNA sequence encoding the ribonucleotide motif or tag; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a

recombinant target nucleotide sequence that encodes a modified RNA of interest comprising the ribonucleotide motif or tag.

415. The method of embodiment 414, wherein ribonucleotide motif or tag is a detection moiety.

416. The method of embodiment 414, wherein the ribonucleotide motif or tag affects the expression level of the RNA of interest.

417. The method of embodiment 414, wherein the ribonucleotide motif or tag affects the transport or subcellular location of the RNA of interest.

418. The method of embodiment 414, wherein the ribonucleotide motif or tag is selected from the group consisting of SV40 type 1, SV40 type 2, SV40 type 3, hGH, BGH, rbGlob, TK, MALAT1 ENE-mascRNA, KSHV PAN ENE, Smbox/U1 snRNA box, U1 snRNA 3' box, tRNA-lysine, broccoli aptamer, spinach aptamer, mango aptamer, HDV ribozyme, and m6A.

419. The method of embodiment 414, wherein the PEgRNA comprises SEQ ID NOs: 18-25.

420. The method of embodiment 414, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

421. The method of embodiment 414, wherein the napDNAbp is a Cas9 nickase (nCas9).

422. The method of embodiment 414, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

423. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method

produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

424. The method of embodiment 423, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, SUMOylation state of the protein of interest.

GROUP A. FUSION PROTEINS, GUIDES, AND METHODS

[1681] Embodiment 1. A fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase.

[1682] Embodiment 2. The fusion protein of embodiment 1, wherein the fusion protein is capable of carrying out genome editing by target-primed reverse transcription in the presence of an extended guide RNA.

[1683] Embodiment 3. The fusion protein of embodiment 1, wherein the napDNAbp has a nickase activity.

[1684] Embodiment 4. The fusion protein of embodiment 1, wherein the napDNAbp is a Cas9 protein or variant thereof.

[1685] Embodiment 5. The fusion protein of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[1686] Embodiment 6. The fusion protein of embodiment 1, wherein the napDNAbp is Cas9 nickase (nCas9).

[1687] Embodiment 7. The fusion protein of embodiment 1, wherein the napDNAbp is selected from the group consisting of: Cas9, CasX, CasY, Cpf1, C2c1, C2c2, C2C3, and Argonaute and optionally has a nickase activity.

[1688] Embodiment 8. The fusion protein of embodiment 1, wherein the fusion protein when complexed with an extended guide RNA is capable of binding to a target DNA sequence.

[1689] Embodiment 9. The fusion protein of embodiment 8, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[1690] Embodiment 10. The fusion protein of embodiment 8, wherein the binding of the fusion protein complexed to the extended guide RNA forms an R-loop.

[1691] Embodiment 11. The fusion protein of embodiment 10, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the extended guide RNA and the target strand, and (ii) the complementary non-target strand.

[1692] Embodiment 12. The fusion protein of embodiment 11, wherein the complementary non-target strand is nicked to form a reverse transcriptase priming sequence having a free 3′ end.

[1693] Embodiment 13. The fusion protein of embodiment 2, wherein the extended guide RNA comprises (a) a guide RNA, and (b) an RNA extension at the 5′ or the 3′ end of the guide RNA, or at an intramolecular location in the guide RNA.

[1694] Embodiment 14. The fusion protein of embodiment 13, wherein the RNA extension comprises (i) a reverse transcription template sequence comprising a desired nucleotide change, (ii) a reverse transcription primer binding site, and (iii) optionally, a linker sequence.

[1695] Embodiment 15. The fusion protein of embodiment 14, wherein the reverse transcription template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

[1696] Embodiment 16. The fusion protein of embodiment 13, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[1697] Embodiment 17. The fusion protein of embodiment 15, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change.

[1698] Embodiment 18. The fusion protein of embodiment 15, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5′ end.

[1699] Embodiment 19. The fusion protein of embodiment 18, wherein the endogenous DNA sequence having the 5′ end is excised by the cell.

[1700] Embodiment 20. The fusion protein of embodiment 18, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[1701] Embodiment 21. The fusion protein of embodiment 14, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence, or wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

[1702] Embodiment 22. The fusion protein of embodiment 1, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence to SEQ ID NO: 18.

[1703] Embodiment 23. The fusion protein of embodiment 1, wherein the napDNAbp comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1704] [0001] The fusion protein of embodiment 1, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89.

[1705] Embodiment 24. The fusion protein of embodiment 1, wherein the reverse transcriptase comrises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1706] [0002] The fusion protein of embodiment 1, wherein the reverse transcriptase comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NO: 89.

[1707] Embodiment 25. The fusion protein of embodiment 1, wherein the reverse transcriptase comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1708] Embodiment 26. The fusion protein of embodiment 1, wherein the reverse transcriptase is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

[1709] Embodiment 27. The fusion protein of any one of the previous embodiments, wherein the fusion protein comprises the structure NH2-[napDNAbp]-[reverse transcriptase]-COOH; or NH2-[reverse transcriptase]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

[1710] Embodiment 28. The fusion protein of embodiment 27, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[1711] Embodiment 29. The fusion protein of embodiment 14, wherein the desired nucleotide change is a single nucleotide change, an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

[1712] Embodiment 30. The fusion protein of embodiment 29, wherein the insert or deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 , at least 23 , at least 24 , at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50.

[1713] Embodiment 31. An extended guide RNA comprising a guide RNA and at least one RNA extension.

[1714] Embodiment 32. The extended guide RNA of embodiment 1, wherein the RNA extension is position at the 3′ or 5′ end of the guide RNA, or at an intramolecular position in the guide RNA.

[1715] Embodiment 33. The extended guide RNA of embodiment 31, wherein the extended guide RNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[1716] Embodiment 34. The extended guide RNA of embodiment 33, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1717] Embodiment 35. The extended guide RNA of embodiment 31, wherein the at least one RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

[1718] Embodiment 36. The extended guide RNA of embodiment 35, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[1719] Embodiment 37. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[1720] Embodiment 38. The extended guide RNA of embodiment 35, wherein the reverse transcription primer binding site sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[1721] Embodiment 39. The extended guide RNA of embodiment 35, wherein the optional linker sequence is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[1722] Embodiment 40. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

[1723] Embodiment 41. The extended guide RNA of embodiment 40, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5′ end in the

target DNA sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

[1724] Embodiment 42. The extended guide RNA of embodiment 41, wherein the endogenous single-stranded DNA having the free 5′ end is excised by the cell.

[1725] Embodiment 43. The extended guide RNA of embodiment 41, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[1726] Embodiment 44. The extended guide RNA of embodiment 31, comprising the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810.

[1727] Embodiment 45. The extended guide RNA of embodiment 35, wherein the reverse transcription template sequence comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[1728] Embodiment 46. The extended guide RNA of embodiment 35, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

[1729] Embodiment 47. The extended guide RNA of embodiment 35, wherein the optional linker sequence is at least 1 nucleotide, or at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 nucleotides in length.

[1730] Embodiment 48. A complex comprising comprising a fusion protein of any one of embodiments 1-30 and an extended guide RNA.

[1731] Embodiment 49. The complex of embodiment 48, wherein the extended guide RNA comprises a guide RNA and an RNA extension at the 3′ or 5′ end of the guide RNA or at an intramolecular position in the guide RNA.

[1732] Embodiment 50. The complex of embodiment 48, wherein the extended guide RNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[1733] Embodiment 51. The complex of embodiment 50, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1734] Embodiment 52. The complex of embodiment 49, wherein the at least one RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

[1735] Embodiment 53. The complex of embodiment 48, wherein the extended guide RNA comprises the nucleotide sequence of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810.

[1736] Embodiment 54. The complex of embodiment 52, wherein the reverse transcription template sequence comprises a nucleotide sequence having at least 80%, or 85%, or 90%, or 95%, or 99% sequence identity with the endogenous DNA target.

[1737] Embodiment 55. The complex of embodiment 52, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

[1738] Embodiment 56. A complex comprising comprising a napDNAbp and an extended guide RNA.

[1739] Embodiment 57. The complex of embodiment 56, wherein the napDNAbp is a Cas9 nickase.

[1740] Embodiment 58. The complex of embodiment 56, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[1741] Embodiment 59. The complex of embodiment 57, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487..

[1742] Embodiment 60. The complex of embodiment 57, wherein the extended guide RNA comprises a guide RNA and an RNA extension at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[1743] Embodiment 61. The complex of embodiment 57, wherein the extended guide RNA is capable of directing the napDNAbp to a target DNA sequence.

[1744] Embodiment 62. The complex of embodiment 61, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the spacer sequence hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1745] Embodiment 63. The complex of embodiment 61, wherein the RNA extension comprises (i) a reverse transcription template sequence, (ii) a reverse transcription primer binding site, and (iii) optionally a linker sequence.

[1746] Embodiment 64. The complex of embodiment 57, wherein the extended guide RNA comprises the nucleotide sequence of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810.

[1747] Embodiment 65. The complex of embodiment 63, wherein the reverse transcription template sequence comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[1748] Embodiment 66. The complex of embodiment 63, wherein the reverse transcription primer binding site hybridizes with a free 3' end of the cut DNA.

[1749] Embodiment 67. The complex of embodiment 63, wherein the optional linker sequence is at least 1 nucleotide, or at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 nucleotides in length.

[1750] Embodiment 68. A polynucleotide encoding the fusion protein of any of embodiments 1-30.

[1751] Embodiment 69. A vector comprising the polynucleotide of embodiment 68.

[1752] Embodiment 70. A cell comprising the fusion protein of any of embodiments 1-30 and an extended guide RNA bound to the napDNAbp of the fusion protein.

[1753] Embodiment 71. A cell comprising a complex of any one of embodiments 48-67.

[1754] Embodiment 72. A pharmaceutical composition comprising: (i) a fusion protein of any of embodiments 1-30, the complex of embodiments 48-67, the polynucleotide of embodiment 68, or the vector of embodiment 69; and (ii) a pharmaceutically acceptable excipient.

[1755] Embodiment 73. A pharmaceutical composition comprising: (i) the complex of embodiments 48-67 (ii) reverse transcriptase provided in trans; and (iii) a pharmaceutically acceptable excipient.

[1756] Embodiment 74. A kit comprising a nucleic acid construct, comprising: (i) a nuleic acid sequencing encoding the fusion protein of any one of embodiments 1-30; and (ii) a promoter that drives expression of the sequence of (i).

[1757] Embodiment 75. A method for installing a desired nucleotide change in a double-stranded DNA sequence, the method comprising:

[1758] (i) contacting the double-stranded DNA sequence with a complex comprising a fusion protein and an extended guide RNA, wherein the fusion protein comprises a napDNAbp and a reverse transcriptase and wherein the extended guide RNA comprises a reverse transcription template sequence comprising the desired nucleotide change;

[1759] (ii) nicking the double-stranded DNA sequence on the non-target strand, thereby generating a free single-strand DNA having a 3′ end;

[1760] (iii) hybridizing the 3′ end of the free single-strand DNA to the reverse transcription template sequence, thereby priming the reverse transcriptase domain;

[1761] (iv) polymerizing a strand of DNA from the 3′ end, thereby generating a single-strand DNA flap comprising the desired nucleotide change;

[1762] (v) replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

[1763] Embodiment 76. The method of embodiment 75, wherein the step of (v) replacing comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

[1764] Embodiment 77. The method of embodiment 76, wherein the desired nucleotide change is a single nucleotide substitution, a deletion, or an insertion.

[1765] Embodiment 78. The method of embodiment 77, wherein the single nucleotide substitution is a transition or a transversion.

[1766] Embodiment 79. The method of embodiment 76, wherein the desired nucleotide change is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[1767] Embodiment 80. The method of embodiment 76, wherein the desired nucleoid change converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[1768] Embodiment 81. The method of embodiment 76, wherein the desired nucleotide change is an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[1769] Embodiment 82. The method of embodiment 76, wherein the desired nucleotide change corrects a disease-associated gene.

[1770] Embodiment 83. The method of embodiment 82, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease.

[1771] Embodiment 84. The method of embodiment 82, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[1772] Embodiment 85. The method of embodiment 76, wherein the napDNAbp is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9.

[1773] Embodiment 86. The method of embodiment 76, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[1774] Embodiment 87. The method of embodiment 76, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1775] Embodiment 88. The method of embodiment 76, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-

122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1776] Embodiment 89. The method of embodiment 76, wherein the reverse transcriptase domain comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1777] Embodiment 90. The method of embodiment 76, wherein the extended guide RNA comprises an RNA extension at the 3` or 5` ends or at an intramolecular location in the guide RNA, wherein the RNA extension comprises the reverse transcription template sequence.

[1778] Embodiment 91. The method of embodiment 90, wherein the RNA extension is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[1779] Embodiment 92. The method of embodiment 76, wherein the extended guide RNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 394, 429-442, 641-649, 678-692, 2997-3103, 3113-3121, 3305-3455, 3479-3493, 3522-3556, 3628-3698, and 3755-3810.

[1780] Embodiment 93. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising:

[1781] (i) contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a guide RNA which targets the napDNAbp to the target locus, wherein the guide RNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change;

[1782] (ii) forming an exposed 3´ end in a DNA strand at the target locus;

[1783] (iii) hybridizing the exposed 3´ end to the RT template sequence to prime reverse transcription;

[1784] (iv) synthesizing a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence by reverse transcriptase;

[1785] (v) and incorporating the at least one desired nucleotide change into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

[1786] Embodiment 94. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a transition.

[1787] Embodiment 95. The method of embodiment 94, wherein the transition is selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[1788] Embodiment 96. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a transversion.

[1789] Embodiment 97. The method of embodiment 96, wherein the transversion is selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[1790] Embodiment 98. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[1791] Embodiment 99. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[1792] Embodiment 100. The method of embodiment 93, wherein the one or more changes in the nucleotide sequence comprises a correction to a disease-associated gene.

[1793] Embodiment 101. The method of embodiment 100, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease.

[1794] Embodiment 102. The method of embodiment 100, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of:

heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[1795] Embodiment 103. The method of embodiment 93, wherein the napDNAbp is a nuclease active Cas9 or variant thereof.

[1796] Embodiment 104. The method of embodiment 93, wherein the napDNAbp is a nuclease inactive Cas9 (dCas9) or Cas9 nickase (nCas9), or a variant thereof.

[1797] Embodiment 105. The method of embodiment 93, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[1798] Embodiment 106. The method of embodiment 93, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1799] Embodiment 107. The method of embodiment 93, wherein the reverse transcriptase is introduced in trans.

[1800] Embodiment 108. The method of embodiment 93, wherein the napDNAbp comprises a fusion to a reverse transcriptase.

[1801] Embodiment 109. The method of embodiment 93, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1802] Embodiment 110. The method of embodiment 93, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1803] Embodiment 111. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises nicking the DNA strand with a nuclease.

[1804] Embodiment 112. The method of embodiment 111, wherein the nuclease is the napDNAbp, is provided as a fusion domain of napDNAbp, or is provided in trans.

[1805] Embodiment 113. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises contacting the DNA strand with a chemical agent.

[1806] Embodiment 114. The method of embodiment 93, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises introducing a replication error.

[1807] Embodiment 115. The method of embodiment 93, wherein the step of contacting the DNA molecule with the napDNAbp and the guide RNA forms an R-loop.

[1808] Embodiment 116. The method of embodiment 115, wherein the DNA strand in which the exposed 3' end is formed is in the R-loop.

[1809] Embodiment 117. The method of embodiment 93, wherein guide RNA comprises an extended portion that comprises the reverse transcriptase (RT) template sequence.

[1810] Embodiment 118. The method of embodiment 117, wherein the extended portion is at the 3´ end of the guide RNA, the 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[1811] Embodiment 119. The method of embodiment 93, wherein the guide RNA further comprises a primer binding site.

[1812] Embodiment 120. The method of embodiment 93, wherein the guide RNA further comprises a spacer sequence.

[1813] Embodiment 121. The method of embodiment 93, wherein the RT template sequence is homologous to the corresponding endogenous DNA.

[1814] Embodiment 122. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change; and (c) incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

[1815] Embodiment 123. The method of embodiment 122, wherein the RT template is located at the 3´ end of the guide RNA, the 5′ end of the guide RNA, or at an intramolecular location in the guide RNA.

[1816] Embodiment 124. The method of embodiment 122, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

[1817] Embodiment 125. The method of clami 122, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[1818] Embodiment 126. The method of clami 122, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[1819] Embodiment 127. The method of embodiment 122, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[1820] Embodiment 128. A polynucleotide encoding the extended guide RNA of any one of embodiments 31-47.

[1821] Embodiment 129. A vector comprising the polynucleotide of embodiment 128.

[1822] Embodiment 130. A cell comprising the vector of embodiment 129.

[1823] Embodiment 131. The fusion protein of any of embodiments 1-30, wherein the reverse transcriptase is an error-prone reverse transcriptase.

[1824] Embodiment 132. A method for mutagenizing a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a mutagenized single strand DNA; and (c) incorporating the mutagenized single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[1825] Embodiment 133. The method of embodiment 132, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1826] Embodiment 134. The method of embodiment 132, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1827] Embodiment 135. The method of embodiment 132, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1828] Embodiment 136. The method of embodiment 132, wherein the guide RNA comprises SEQ ID NOs: 222.

[1829] Embodiment 137. The method of embodiment 132, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3′ end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

[1830] Embodiment 138. A method for replacing a trinucleotide repeat expansion mutation in a target DNA molecule with a healthy sequence comprising a healthy number of repeat trinucleotides, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising the replacement sequence, wherein said fusion protein intr; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the replacement sequence; and (c) incorporating the single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[1831] Embodiment 139. The method of embodiment 138, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1832] Embodiment 140. The method of embodiment 138, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1833] Embodiment 141. The method of embodiment 138, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1834] Embodiment 142. The method of embodiment 138, wherein the guide RNA comprises SEQ ID NOs: 222.

[1835] Embodiment 143. The method of embodiment 138, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3′ end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

[1836] Embodiment 144. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation is associated with Huntington's Disease, Fragile X syndrome, or Friedreich's ataxia.

[1837] Embodiment 145. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of CAG triplets.

[1838] Embodiment 146. The method of embodiment 138, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of GAA triplets.

[1839] Embodiment 147. A method of installing a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the functional moiety; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the functional moiety.

[1840] Embodiment 148. The method of embodiment 147, wherein functional moiety is peptide tag.

[1841] Embodiment 149. The method of embodiment 148, wherein the peptide tag is an affinity tag, solubilization tag, chromatography tag, epitope tag, or a fluorescence tag.

[1842] Embodiment 150. The method of embodiment 148, wherein the peptide tag is selected from the group consisting of: AviTag (SEQ ID NO: 245); C-tag (SEQ ID NO: 246); Calmodulin-tag (SEQ ID NO: 247); polyglutamate tag (SEQ ID NO: 248); E-tag (SEQ ID NO: 249); FLAG-tag (SEQ ID NO: 2); HA-tag (SEQ ID NO: 5); His-tag (SEQ ID NOs: 252-262); Myc-tag (SEQ ID NO: 6); NE-tag (SEQ ID NO: 264); Rho1D4-tag (SEQ ID NO: 265); S-tag (SEQ ID NO: 266); SBP-tag (SEQ ID NO: 267); Softag-1 (SEQ ID NO: 268); Softag-2 (SEQ ID NO: 269); Spot-tag (SEQ ID NO: 270); Strep-tag (SEQ ID NO: 271); TC tag (SEQ ID NO: 272); Ty tag (SEQ ID NO: 273); V5 tag (SEQ ID NO: 3); VSV-tag (SEQ ID NO: 275); and Xpress tag (SEQ ID NO: 276).

[1843] Embodiment 151. The method of embodiment 148, wherein the peptide tag is selected from the group consisting of: AU1 epitope (SEQ ID NO: 278); AU5 epitope (SEQ ID NO: 279); Bacteriophage T7 epitope (T7-tag) (SEQ ID NO: 280); Bluetongue virus tag

(B-tag) (SEQ ID NO: 281); E2 epitope (SEQ ID NO: 282); Histidine affinity tag (HAT) (SEQ ID NO: 283); HSV epitope (SEQ ID NO: 284); Polyarginine (Arg-tag) (SEQ ID NO: 285); Polyaspartate (Asp-tag) (SEQ ID NO: 286); Polyphenylalanine (Phe-tag) (SEQ ID NO: 287); S1-tag (SEQ ID NO: 288); S-tag (SEQ ID NO: 266); and VSV-G (SEQ ID NO: 275).

[1844] Embodiment 152. The method of embodiment 147, wherein the functional moiety is an immunoepitope.

[1845] Embodiment 153. The method of embodiment 152, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 398); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

[1846] Embodiment 154. The method of embodiment 147, wherein the functional moiety alters the localization of the protein of interest.

[1847] Embodiment 155. The method of embodiment 147, wherein the functional moiety is a degradation tag such that the degradation rate of the protein of interest is altered.

[1848] Embodiment 156. The method of embodiment 155, wherein the degradation tag comprises an amino acid sequence encoding the degradation tags as disclosed herein.

[1849] Embodiment 157. The method of embodiment 147, wherein the functional moiety is a small molecule binding domain.

[1850] Embodiment 158. The method of embodiment 157, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

[1851] Embodiment 159. The method of embodiment 157, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

[1852] Embodiment 160. The method of embodiment 157, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 492-494.

[1853] Embodiment 161. The method of embodiment 157, wherein the small molecule binding domain is installed in two or more proteins of interest.

[1854] Embodiment 162. The method of embodiment 161, wherein the two or more proteins of interest may dimerize upon contacting with a small molecule.

[1855] Embodiment 163. The method of embodiment 157, wherein the small molecule is a dimer of a small molecule selected from the group consisting of those compounds disclosed in Embodiment 163 of Group 1. .

[1856] Embodiment 164. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

[1857] Embodiment 165. The method of embodiment 164, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

[1858] Embodiment 166. The method of embodiment 164, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1859] Embodiment 167. The method of embodiment 164, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1860] Embodiment 168. The method of embodiment 164, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1861] Embodiment 169. The method of embodiment 164, wherein the guide RNA comprises SEQ ID NOs: 222.

[1862] Embodiment 170. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the small molecule dimerization domain.

[1863] Embodiment 171. The method of embodiment 170, further comprising conducting the method on a second protein of interest.

[1864] Embodiment 172. The method of embodiment 171, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

[1865] Embodiment 173. The method of embodiment 170, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

[1866] Embodiment 174. The method of embodiment 170, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

[1867] Embodiment 175. The method of embodiment 170, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 490 and 493-494.

[1868] Embodiment 176. The method of embodiment 170, wherein the small molecule is a dimer of a small molecule selected from the group consisting of those compounds disclosed in Embodiment 163 of Group 1.

[1869] Embodiment 177. The method of embodiment 170, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1870] Embodiment 178. The method of embodiment 170, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1871] Embodiment 179. The method of embodiment 170, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1872] Embodiment 180. The method of embodiment 170, wherein the guide RNA comprises SEQ ID NOs: 222.

[1873] Embodiment 181. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

[1874] Embodiment 182. The method of embodiment 181, wherein the peptide tag is used for purification and/or detection of the protein.

[1875] Embodiment 183. The method of embodiment 181, wherein the peptide tag is a poly-histidine (e.g., HHHHHH) (SEQ ID NO: 252-262), FLAG (e.g., DYKDDDDK) (SEQ ID NO: 2), V5 (e.g., GKPIPNPLLGLDST) (SEQ ID NO: 3), GCN4, HA (e.g., YPYDVPDYA) (SEQ ID NO: 5), Myc (e.g. EQKLISEED) (SEQ ID NO: 6), GST….etc.

[1876] Embodiment 184. The method of embodiment 181, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 1-6, 245-249, 252-262, 264-273, 275-276, 281, 278-288, and 622.

[1877] Embodiment 185. The method of embodiment 181, wherein the peptide tag is fused to the protein by a linker.

[1878] Embodiment 186. The method of embodiment 181, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

[1879] Embodiment 187. The method of embodiment 181, wherein the linker has an amino acid sequence of SEQ ID NO: 127, 165-176, 446, 453, and 767-769.

[1880] Embodiment 188. The method of embodiment 181, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[1881] Embodiment 189. The method of embodiment 181, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[1882] Embodiment 190. The method of embodiment 181, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[1883] Embodiment 191. A method of preventing or halting the progression of a prion disease by installing on or more protective mutations into PRNP encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the protective mutation; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a PRNP comprising a protective mutation and which is resistant to misfolding.

[1884] Embodiment 192. The method of embodiment 191, wherein the prion disease is a human prion disease.

[1885] Embodiment 193. The method of embodiment 191, wherein the prion disease is an animal prion disease.

[1886] Embodiment 194. The method of embodiment 192, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, or Kuru.

[1887] Embodiment 195. The method of embodiment 193, wherein the prion disease is Bovine Spongiform Encephalopathy (BSE or "mad cow disease"), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy.

[1888] Embodiment 196. The method of embodiment 191, wherein the wildtype PRNP amino acid sequence is SEQ ID NOs: 291-292.

[1889] Embodiment 197. The method of embodiment 191, wherein the method results in a modified PRNP amino acid sequence selected from the group consisting of SEQ ID NOs: 293-309, and 311-323, wherein said modified PRNP protein is resistant to misfolding.

[1890] Embodiment 198. The method of embodiment 191, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1891] Embodiment 199. The method of embodiment 191, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1892] Embodiment 200. The method of embodiment 191, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1893] Embodiment 201. The method of embodiment 191, wherein the guide RNA comprises SEQ ID NOs: 222.

[1894] Embodiment 202. A method of installing a ribonucleotide motif or tag in an RNA of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the ribonucleotide motif or tag; (b) polymerizing a single strand DNA sequence encoding the ribonucleotide motif or tag; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified RNA of interest comprising the ribonucleotide motif or tag.

[1895] Embodiment 203. The method of embodiment 202, wherein ribonucleotide motif or tag is a detection moiety.

[1896] Embodiment 204. The method of embodiment 202, wherein the ribonucleotide motif or tag affects the expression level of the RNA of interest.

[1897] Embodiment 205. The method of embodiment 202, wherein the ribonucleotide motif or tag affects the transport or subcellular location of the RNA of interest.

[1898] Embodiment 206. The method of embodiment 202, wherein the ribonucleotide motif or tag is selected from the group consisting of SV40 type 1, SV40 type 2, SV40 type 3, hGH, BGH, rbGlob, TK, MALAT1 ENE-mascRNA, KSHV PAN ENE, Smbox/U1 snRNA box, U1 snRNA 3' box, tRNA-lysine, broccoli aptamer, spinach aptamer, mango aptamer, HDV ribozyme, and m6A.

[1899] Embodiment 207. The method of embodiment 202, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[1900] Embodiment 208. The method of embodiment 202, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[1901] Embodiment 209. The method of embodiment 202, wherein the napDNAbp is a Cas9 nickase (nCas9).

[1902] Embodiment 210. The method of embodiment 202, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1903] Embodiment 211. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

[1904] Embodiment 212. The method of embodiment 211, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, SUMOylation state of the protein of interest.

[1905] Embodiment 213. A fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase.

[1906] Embodiment 214. The fusion protein of embodiment 213, wherein the fusion protein is capable of carrying out prime editing in the presence of an prime editing guide RNA (PEgRNA).

[1907] Embodiment 215. The fusion protein of embodiment 213, wherein the napDNAbp has a nickase activity.

[1908] Embodiment 216. The fusion protein of embodiment 213, wherein the napDNAbp is a Cas9 protein or variant thereof.

[1909] Embodiment 217. The fusion protein of embodiment 213, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[1910] Embodiment 218. The fusion protein of embodiment 213, wherein the napDNAbp is Cas9 nickase (nCas9).

[1911] Embodiment 219. The fusion protein of embodiment 213, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[1912] Embodiment 220. The fusion protein of embodiment 213, wherein the fusion protein when complexed with a PEgRNA is capable of binding to a target DNA sequence.

[1913] Embodiment 221. The fusion protein of embodiment 220, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[1914] Embodiment 222. The fusion protein of embodiment 220, wherein the binding of the fusion protein complexed to the PEgRNA forms an R-loop.

[1915] Embodiment 223. The fusion protein of embodiment 222, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the PEgRNA and the target strand, and (ii) the complementary non-target strand.

[1916] Embodiment 224. The fusion protein of embodiment 223, wherein the complementary non-target strand is nicked to form a priming sequence having a free 3´ end.

[1917] Embodiment 225. The fusion protein of embodiment 214, wherein the PEgRNA comprises (a) a guide RNA and (b) an extension arm at the 5´ or the 3´ end of the guide RNA, or at an intramolecular location in the guide RNA.

[1918] Embodiment 226. The fusion protein of embodiment 225, wherein the extension arm comprises (i) a DNA synthesis template sequence comprising a desired nucleotide change, and (ii) a primer binding site.

[1919] Embodiment 227. The fusion protein of embodiment 226, wherein the DNA synthesis template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

[1920] Embodiment 228. The fusion protein of embodiment 225, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[1921] Embodiment 229. The fusion protein of embodiment 227, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change.

[1922] Embodiment 230. The fusion protein of embodiment 227, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5' end.

[1923] Embodiment 231. The fusion protein of embodiment 230, wherein the endogenous DNA sequence having the 5´ end is excised by the cell.

[1924] Embodiment 232. The fusion protein of embodiment 230, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[1925] Embodiment 233. The fusion protein of embodiment 226, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence, or wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

[1926] Embodiment 234. The fusion protein of embodiment 213, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence to SEQ ID NO: 18.

[1927] Embodiment 235. The fusion protein of embodiment 213, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1928] Embodiment 236. The fusion protein of embodiment 213, wherein the polymerase is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1929] Embodiment 237. The fusion protein of embodiment 213, wherein the polymerase is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1930] Embodiment 238. The fusion protein of embodiment 213, wherein the polymerase is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

[1931] Embodiment 239. The fusion protein of any one of the previous embodiments, wherein the fusion protein comprises the structure NH2-[napDNAbp]-[polymerase]-COOH; or NH2-[polymerase]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

[1932] Embodiment 240. The fusion protein of embodiment 239, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[1933] Embodiment 241. The fusion protein of embodiment 226, wherein the desired nucleotide change is a single nucleotide change, an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

[1934] Embodiment 242. The fusion protein of embodiment 241, wherein the insert or deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 , at least 23 , at least 24 , at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50.

[1935] Embodiment 243. A PEgRNA comprising a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template.

[1936] Embodiment 244. The PEgRNA of embodiment 241, wherein the nucleic acid extension arm is position at the 3′ or 5′ end of the guide RNA, or at an intramolecular position in the guide RNA, and wherein the nucleic acid extension arm is DNA or RNA.

[1937] Embodiment 245. The PEgRNA of embodiment 242, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[1938] Embodiment 246. The PEgRNA of embodiment 245, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1939] Embodiment 247. The PEgRNA of embodiment 243, wherein the at least one nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

[1940] Embodiment 248. The PEgRNA of embodiment 247, wherein the nucleic acid extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[1941] Embodiment 249. The PEgRNA of embodiment 247, wherein the DNA synthesis template is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[1942] Embodiment 250. The PEgRNA of embodiment 247, wherein the primer binding site is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[1943] Embodiment 251. The PEgRNA of embodiment 243, further comprising at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[1944] Embodiment 252. The PEgRNA of embodiment 247, wherein the DNA synthesis template encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

[1945] Embodiment 253. The PEgRNA of embodiment 252, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5′ end in the target DNA

sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

[1946] Embodiment 254. The PEgRNA of embodiment 253, wherein the endogenous single-stranded DNA having the free 5´ end is excised by the cell.

[1947] Embodiment 255. The PEgRNA of embodiment 253, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[1948] Embodiment 256. The PEgRNA of embodiment 243, comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[1949] Embodiment 257. The PEgRNA of embodiment 247, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[1950] Embodiment 258. The PEgRNA of embodiment 247, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[1951] Embodiment 259. The PEgRNA of embodiment 251, wherein the at least one additional structure is located at the 3' or 5' end of the PEgRNA.

[1952] Embodiment 260. A complex comprising comprising a fusion protein of any one of embodiments 213-242 and an PEgRNA.

[1953] Embodiment 261. The complex of embodiment 260, wherein the PEgRNA comprises a guide RNA and an nucleic acid extension arm at the 3´ or 5´ end of the guide RNA or at an intramolecular position in the guide RNA.

[1954] Embodiment 262. The complex of embodiment 260, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[1955] Embodiment 263. The complex of embodiment 262, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1956] Embodiment 264. The complex of embodiment 261, wherein the at least one nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

[1957] Embodiment 265. The complex of embodiment 260, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[1958] Embodiment 266. The complex of embodiment 264, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[1959] Embodiment 267. The complex of embodiment 264, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[1960] Embodiment 268. A complex comprising comprising a napDNAbp and an PEgRNA.

[1961] Embodiment 269. The complex of embodiment 268, wherein the napDNAbp is a Cas9 nickase.

[1962] Embodiment 270. The complex of embodiment 268, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[1963] Embodiment 271. The complex of embodiment 268, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1964] Embodiment 272. The complex of embodiment 268, wherein the PEgRNA comprises a guide RNA and a nucleic acid extension arm at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[1965] Embodiment 273. The complex of embodiment 268, wherein the PEgRNA is capable of directing the napDNAbp to a target DNA sequence.

[1966] Embodiment 274. The complex of embodiment 272, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the

spacer sequence of the PEgRNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[1967] Embodiment 275. The complex of embodiment 273, wherein the nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

[1968] Embodiment 276. The complex of embodiment 269, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[1969] Embodiment 277. The complex of embodiment 276, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[1970] Embodiment 278. The complex of embodiment 276, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[1971] Embodiment 279. The complex of embodiment 276, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[1972] Embodiment 280. A polynucleotide encoding the fusion protein of any of embodiments 213-242.

[1973] Embodiment 281. A vector comprising the polynucleotide of embodiment 280.

[1974] Embodiment 282. A cell comprising the fusion protein of any of embodiments 213-242 and an PEgRNA bound to the napDNAbp of the fusion protein.

[1975] Embodiment 283. A cell comprising a complex of any one of embodiments 260-279.

[1976] Embodiment 284. A pharmaceutical composition comprising: (i) a fusion protein of any of embodiments 213-242, the complex of embodiments 260-279, the polynucleotide of embodiment 68, or the vector of embodiment 69; and (ii) a pharmaceutically acceptable excipient.

[1977] Embodiment 285. A pharmaceutical composition comprising: (i) the complex of embodiments 260-279 (ii) a polymerase provided in trans; and (iii) a pharmaceutically acceptable excipient.

[1978] Embodiment 286. A kit comprising a nucleic acid construct, comprising: (i) a nuleic acid sequencing encoding the fusion protein of any one of embodiments 213-242; and (ii) a promoter that drives expression of the sequence of (i).

[1979] Embodiment 287. A method for installing a desired nucleotide change in a double-stranded DNA sequence, the method comprising:

[1980] (i) contacting the double-stranded DNA sequence with a complex comprising a fusion protein and a PEgRNA, wherein the fusion protein comprises a napDNAbp and a polymerase and wherein the PEgRNA comprises a DNA synthesis template comprising the desired nucleotide change and a primer binding site;

[1981] (ii) nicking the double-stranded DNA sequence, thereby generating a free single-strand DNA having a 3´ end;

[1982] (iii) hybridizing the 3` end of the free single-strand DNA to the primer binding site, thereby priming the polymerase;

[1983] (iv) polymerizing a strand of DNA from the 3´ end hybridized to the primer binding site, thereby generating a single-strand DNA flap comprising the desired nucleotide change and which is complementary to the DNA synthesis template;

[1984] (v) replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

[1985] Embodiment 288. The method of embodiment 287, wherein the step of (v) replacing comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

[1986] Embodiment 289. The method of embodiment 288, wherein the desired nucleotide change is a single nucleotide substitution, a deletion, or an insertion.

[1987] Embodiment 290. The method of embodiment 289, wherein the single nucleotide substitution is a transition or a transversion.

[1988] Embodiment 291. The method of embodiment 288, wherein the desired nucleotide change is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[1989] Embodiment 292. The method of embodiment 288, wherein the desired nucleoid change converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[1990] Embodiment 293. The method of embodiment 288, wherein the desired nucleotide change is an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[1991] Embodiment 294. The method of embodiment 288, wherein the desired nucleotide change corrects a disease-associated gene.

[1992] Embodiment 295. The method of embodiment 294, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

[1993] Embodiment 296. The method of embodiment 294, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[1994] Embodiment 297. The method of embodiment 287, wherein the napDNAbp is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9.

[1995] Embodiment 298. The method of embodiment 287, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 18.

[1996] Embodiment 299. The method of embodiment 287, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99%

sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[1997] Embodiment 300. The method of embodiment 287, wherein the polymerase is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1998] Embodiment 301. The method of embodiment 287, wherein the polymerase is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[1999] Embodiment 302. The method of embodiment 287, wherein the PEgRNA comprises a nucleic acid extension arm at the 3` or 5` ends or at an intramolecular location in the guide RNA, wherein the extension arm comprises the DNA synthesis template sequence and the primer binding site.

[2000] Embodiment 303. The method of embodiment 302, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[2001] Embodiment 304. The method of embodiment 287, wherein the PEgRNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2002] Embodiment 305. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising:

[2003] (i) contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a PEgRNA which targets the napDNAbp to the target locus, wherein the PEgRNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change and a primer binding site;

[2004] (ii) forming an exposed 3´ end in a DNA strand at the target locus;

[2005] (iii) hybridizing the exposed 3´ end to the primer binding site to prime reverse transcription;

[2006] (iv) synthesizing a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence by reverse transcriptase;

[2007] (v) and incorporating the at least one desired nucleotide change into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

[2008] Embodiment 306. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a transition.

[2009] Embodiment 307. The method of embodiment 306, wherein the transition is selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[2010] Embodiment 308. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a transversion.

[2011] Embodiment 309. The method of embodiment 308, wherein the transversion is selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[2012] Embodiment 310. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (9) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2013] Embodiment 311. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[2014] Embodiment 312. The method of embodiment 305, wherein the one or more changes in the nucleotide sequence comprises a correction to a disease-associated gene.

[2015] Embodiment 313. The method of embodiment 312, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1;

Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

[2016] Embodiment 314. The method of embodiment 312, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[2017] Embodiment 315. The method of embodiment 305, wherein the napDNAbp is a nuclease active Cas9 or variant thereof.

[2018] Embodiment 316. The method of embodiment 305, wherein the napDNAbp is a nuclease inactive Cas9 (dCas9) or Cas9 nickase (nCas9), or a variant thereof.

[2019] Embodiment 317. The method of embodiment 305, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[2020] Embodiment 318. The method of embodiment 305, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2021] Embodiment 319. The method of embodiment 305, wherein the reverse transcriptase is introduced in trans.

[2022] Embodiment 320. The method of embodiment 305, wherein the napDNAbp comprises a fusion to a reverse transcriptase.

[2023] Embodiment 321. The method of embodiment 305, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2024] Embodiment 322. The method of embodiment 305, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2025] Embodiment 323. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises nicking the DNA strand with a nuclease.

[2026] Embodiment 324. The method of embodiment 323, wherein the nuclease is provided is provided in trans.

[2027] Embodiment 325. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises contacting the DNA strand with a chemical agent.

[2028] Embodiment 326. The method of embodiment 305, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises introducing a replication error.

[2029] Embodiment 327. The method of embodiment 305, wherein the step of contacting the DNA molecule with the napDNAbp and the guide RNA forms an R-loop.

[2030] Embodiment 328. The method of embodiment 327, wherein the DNA strand in which the exposed 3' end is formed is in the R-loop.

[2031] Embodiment 329. The method of embodiment 315, wherein the PEgRNA comprises an extension arm that comprises the reverse transcriptase (RT) template sequence and the primer binding site.

[2032] Embodiment 330. The method of embodiment 329, wherein the extension arm is at the 3´ end of the guide RNA, the 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[2033] Embodiment 331. The method of embodiment 305, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[2034] Embodiment 332. The method of embodiment 305, wherein the PEgRNA further comprises a homology arm.

[2035] Embodiment 333. The method of embodiment 305, wherein the RT template sequence is homologous to the corresponding endogenous DNA.

[2036] Embodiment 334. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template

comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change; and (c) incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2037] Embodiment 335. The method of embodiment 334, wherein the RT template is located at the 3´ end of the guide RNA, the 5' end of the guide RNA, or at an intramolecular location in the guide RNA.

[2038] Embodiment 336. The method of embodiment 334, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

[2039] Embodiment 337. The method of embodiment 334, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[2040] Embodiment 338. The method of clami 334, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[2041] Embodiment 339. The method of embodiment 334, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2042] Embodiment 340. A polynucleotide encoding the PEgRNA of any one of embodiments 243-259.

[2043] Embodiment 341. A vector comprising the polynucleotide of embodiment 340.

[2044] Embodiment 342. A cell comprising the vector of embodiment 341.

[2045] Embodiment 343. The fusion protein of embodiment 213, wherein the polymerase is an error-prone reverse transcriptase.

[2046] Embodiment 344. A method for mutagenizing a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone reverse transcriptase and (ii) a guide

RNA comprising an RT template comprising a desired nucleotide change; (b) conducting target-primed reverse transcription of the RT template to generate a mutagenized single strand DNA; and (c) incorporating the mutagenized single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2047] Embodiment 345. The method of any prior embodiment, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2048] Embodiment 346. The method of any prior embodiment, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2049] Embodiment 347. The method of embodiment 344, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-25.

[2050] Embodiment 348. The method of embodiment 344, wherein the guide RNA comprises SEQ ID NOs: 222.

[2051] Embodiment 349. The method of embodiment 344, wherein the step of (b) conducting target-primed reverse transcription comprises generating a 3' end primer binding sequence at the target locus that is capable of priming reverse transcription by annealing to a primer binding site on the guide RNA.

[2052] Embodiment 350. A method for replacing a trinucleotide repeat expansion mutation in a target DNA molecule with a healthy sequence comprising a healthy number of repeat trinucleotides, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising DNA synthesis template comprising the replacement sequence and a primer binding site; (b) conducting prime editing to generate a single strand DNA comprising the replacement sequence; and (c) incorporating the single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2053] Embodiment 351. The method of embodiment 350, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2054] Embodiment 352. The method of embodiment 350, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2055] Embodiment 353. The method of embodiment 350, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2056] Embodiment 354. The method of embodiment 350, wherein the guide RNA comprises SEQ ID NOs: 222.

[2057] Embodiment 355. The method of embodiment 350, wherein the step of (b) conducting prime editing comprises generating a 3' end primer binding sequence at the target locus that is capable of priming polymerase by annealing to the primer binding site on the guide RNA.

[2058] Embodiment 356. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation is associated with Huntington's Disease, Fragile X syndrome, or Friedreich's ataxia.

[2059] Embodiment 357. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of CAG triplets.

[2060] Embodiment 358. The method of embodiment 350, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of GAA triplets.

[2061] Embodiment 359. A method of installing a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising DNA synthesis template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the functional moiety; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the functional moiety.

[2062] Embodiment 360. The method of embodiment 359, wherein functional moiety is peptide tag.

[2063] Embodiment 361. The method of embodiment 360, wherein the peptide tag is an affinity tag, solubilization tag, chromatography tag, epitope or immunoepitope tag, or a fluorescence tag.

[2064] Embodiment 362. The method of embodiment 360, wherein the peptide tag is selected from the group consisting of: AviTag (SEQ ID NO: 245); C-tag (SEQ ID NO: 246); Calmodulin-tag (SEQ ID NO: 247); polyglutamate tag (SEQ ID NO: 248); E-tag (SEQ ID NO: 249); FLAG-tag (SEQ ID NO: 2); HA-tag (SEQ ID NO: 5); His-tag (SEQ ID NOs: 252-262); Myc-tag (SEQ ID NO: 6); NE-tag (SEQ ID NO: 264); Rho1D4-tag (SEQ ID NO: 265);

S-tag (SEQ ID NO: 266); SBP-tag (SEQ ID NO: 267); Softag-1 (SEQ ID NO: 268); Softag-2 (SEQ ID NO: 269); Spot-tag (SEQ ID NO: 270); Strep-tag (SEQ ID NO: 271); TC tag (SEQ ID NO: 272); Ty tag (SEQ ID NO: 273); V5 tag (SEQ ID NO: 3); VSV-tag (SEQ ID NO: 275); and Xpress tag (SEQ ID NO: 276).

[2065] Embodiment 363. The method of embodiment 360, wherein the peptide tag is selected from the group consisting of: AU1 epitope (SEQ ID NO: 278); AU5 epitope (SEQ ID NO: 279); Bacteriophage T7 epitope (T7-tag) (SEQ ID NO: 280); Bluetongue virus tag (B-tag) (SEQ ID NO: 281); E2 epitope (SEQ ID NO: 282); Histidine affinity tag (HAT) (SEQ ID NO: 283); HSV epitope (SEQ ID NO: 284); Polyarginine (Arg-tag) (SEQ ID NO: 285); Polyaspartate (Asp-tag) (SEQ ID NO: 286); Polyphenylalanine (Phe-tag) (SEQ ID NO: 287); S1-tag (SEQ ID NO: 288); S-tag (SEQ ID NO: 266); and VSV-G (SEQ ID NO: 275).

[2066] Embodiment 364. The method of embodiment 359, wherein the functional moiety is an immunoepitope.

[2067] Embodiment 365. The method of embodiment 364, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

[2068] Embodiment 366. The method of embodiment 359, wherein the functional moiety alters the localization of the protein of interest.

[2069] Embodiment 367. The method of embodiment 359, wherein the functional moiety is a degradation tag such that the degradation rate of the protein of interest is altered.

[2070] Embodiment 368. The method of embodiment 367, wherein the degradation tag results in the elimination of the tagged protein.

[2071] Embodiment 369. The method of embodiment 359, wherein the functional moiety is a small molecule binding domain.

[2072] Embodiment 370. The method of embodiment 359, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

[2073] Embodiment 371. The method of embodiment 359, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

[2074] Embodiment 372. The method of embodiment 359, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 490 and 493-494.

[2075] Embodiment 373. The method of embodiment 359, wherein the small molecule binding domain is installed in two or more proteins of interest.

[2076] Embodiment 374. The method of embodiment 373, wherein the two or more proteins of interest may dimerize upon contacting with a small molecule.

[2077] Embodiment 375. The method of embodiment 369, wherein the small molecule is a dimer of a small molecule selected from the group consisting of those compounds disclosed in Embodiment 163 of Group 1.

[2078] Embodiment 376. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

[2079] Embodiment 377. The method of embodiment 376, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I

and class II HLA (SEQ ID NO: 424); neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

[2080] Embodiment 378. The method of embodiment 376, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2081] Embodiment 379. The method of embodiment 376, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2082] Embodiment 380. The method of embodiment 376, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2083] Embodiment 381. The method of embodiment 376, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2084] Embodiment 382. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain; (b) polymerizing a single strand DNA sequence encoding the immunoepitope; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the small molecule dimerization domain.

[2085] Embodiment 383. The method of embodiment 382, further comprising conducting the method on a second protein of interest.

[2086] Embodiment 384. The method of embodiment 383, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

[2087] Embodiment 385. The method of embodiment 382, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

[2088] Embodiment 386. The method of embodiment 382, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

[2089] Embodiment 387. The method of embodiment 382, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 490 and 493-494.

[2090] Embodiment 388. The method of embodiment 382, wherein the small molecule is a dimer of a small molecule selected from the group consisting of those compounds disclosed in Embodiment 163 of Group 1.

[2091] Embodiment 389. The method of embodiment 382, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2092] Embodiment 390. The method of embodiment 382, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2093] Embodiment 391. The method of embodiment 382, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2094] Embodiment 392. The method of embodiment 382, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2095] Embodiment 393. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

[2096] Embodiment 394. The method of embodiment 383, wherein the peptide tag is used for purification and/or detection of the protein.

[2097] Embodiment 395. The method of embodiment 383, wherein the peptide tag is a poly-histidine (e.g., HHHHHH) (SEQ ID NO: 252-262), FLAG (e.g., DYKDDDDK) (SEQ ID NO: 2), V5 (e.g., GKPIPNPLLGLDST) (SEQ ID NO: 3), GCN4, HA (e.g., YPYDVPDYA) (SEQ ID NO: 5), Myc (e.g. EQKLISEED) (SEQ ID NO: 6), or GST.

[2098] Embodiment 396. The method of embodiment 383, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 1-6, 245-249, 252-262, 264-273, 275-276, 281, 278-288, and 622.

[2099] Embodiment 397. The method of embodiment 383, wherein the peptide tag is fused to the protein by a linker.

[2100] Embodiment 398. The method of embodiment 383, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

[2101] Embodiment 399. The method of embodiment 383, wherein the linker has an amino acid sequence of SEQ ID NO: 127, 165-176, 446, 453, and 767-769.

[2102] Embodiment 400. The method of embodiment 383, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2103] Embodiment 401. The method of embodiment 383, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[2104] Embodiment 402. The method of embodiment 383, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[2105] Embodiment 403. A method of preventing or halting the progression of a prion disease by installing on or more protective mutations into PRNP encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety; (b) polymerizing a single strand DNA sequence encoding the protective mutation; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a PRNP comprising a protective mutation and which is resistant to misfolding.

[2106] Embodiment 404. The method of embodiment 403, wherein the prion disease is a human prion disease.

[2107] Embodiment 405. The method of embodiment 403, wherein the prion disease is an animal prion disease.

[2108] Embodiment 406. The method of embodiment 404, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, or Kuru.

[2109] Embodiment 407. The method of embodiment 403, wherein the prion disease is Bovine Spongiform Encephalopathy (BSE or "mad cow disease"), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy.

[2110] Embodiment 408. The method of embodiment 403, wherein the wildtype PRNP amino acid sequence is SEQ ID NOs: 291-292.

[2111] Embodiment 409. The method of embodiment 403, wherein the method results in a modified PRNP amino acid sequence selected from the group consisting of SEQ ID NOs: 293-309, 311-323, wherein said modified PRNP protein is resistant to misfolding.

[2112] Embodiment 410. The method of embodiment 403, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2113] Embodiment 411. The method of embodiment 403, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2114] Embodiment 412. The method of embodiment 403, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2115] Embodiment 413. The method of embodiment 403, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2116] Embodiment 414. A method of installing a ribonucleotide motif or tag in an RNA of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the ribonucleotide motif or tag; (b) polymerizing a single strand DNA sequence encoding the ribonucleotide motif or tag; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified RNA of interest comprising the ribonucleotide motif or tag.

[2117] Embodiment 415. The method of embodiment 414, wherein ribonucleotide motif or tag is a detection moiety.

[2118] Embodiment 416. The method of embodiment 414, wherein the ribonucleotide motif or tag affects the expression level of the RNA of interest.

[2119] Embodiment 417. The method of embodiment 414, wherein the ribonucleotide motif or tag affects the transport or subcellular location of the RNA of interest.

[2120] Embodiment 418. The method of embodiment 414, wherein the ribonucleotide motif or tag is selected from the group consisting of SV40 type 1, SV40 type 2, SV40 type 3, hGH, BGH, rbGlob, TK, MALAT1 ENE-mascRNA, KSHV PAN ENE, Smbox/U1 snRNA box, U1 snRNA 3' box, tRNA-lysine, broccoli aptamer, spinach aptamer, mango aptamer, HDV ribozyme, and m6A.

[2121] Embodiment 419. The method of embodiment 414, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2122] Embodiment 420. The method of embodiment 414, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2123] Embodiment 421. The method of embodiment 414, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2124] Embodiment 422. The method of embodiment 414, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2125] Embodiment 423. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

[2126] Embodiment 424. The method of embodiment 423, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, SUMOylation state of the protein of interest.

[2127] Embodiment 425. A fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) domain and a domain comprising an RNA-dependent DNA polymerase activity.

[2128] Embodiment 426. The fusion protein of embodiment 425, wherein the fusion protein is capable of carrying out prime editing in the presence of an prime editing guide RNA (PEgRNA) to install a desired nucleotide change in a target sequence.

[2129] Embodiment 427. The fusion protein of embodiment 425, wherein the napDNAbp domain has a nickase activity.

[2130] Embodiment 428. The fusion protein of embodiment 425, wherein the napDNAbp domain is a Cas9 protein orvariant thereof.

[2131] Embodiment 429. The fusion protein of embodiment 425, wherein the napDNAbp domain is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2132] Embodiment 430. The fusion protein of embodiment 425, wherein the napDNAbp domain is Cas9 nickase (nCas9).

[2133] Embodiment 431. The fusion protein of embodiment 425, wherein the napDNAbp domain is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2134] Embodiment 432. The fusion protein of embodiment 425, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2135] Embodiment 433. The fusion protein of embodiment 425, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766, and optionally wherein the domain comprising an RNA-dependent DNA polymerase is error-prone.

[2136] Embodiment 434. The fusion protein of embodiment 425, wherein the domain comprising an RNA-dependent DNA polymerase activity is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

[2137] Embodiment 435. The fusion protein of embodiment 425, wherein the fusion protein when complexed with a PEgRNA is capable of binding to a target DNA sequence.

[2138] Embodiment 436. The fusion protein of embodiment 435, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[2139] Embodiment 437. The fusion protein of embodiment 435, wherein the binding of the fusion protein complexed to the PEgRNA forms an R-loop.

[2140] Embodiment 438. The fusion protein of embodiment 437, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the PEgRNA and the target strand, and (ii) the complementary non-target strand.

[2141] Embodiment 439. The fusion protein of embodiment 437, wherein the target or the complementary non-target strand is nicked to form a priming sequence having a free 3′ end.

[2142] Embodiment 440. The fusion protein of embodiment 439, wherein the nick site is upstream of the PAM sequence on the target strand.

[2143] Embodiment 441. The fusion protein of embodiment 439, wherein the nick site is upstream of the PAM sequence on the non-target strand.

[2144] Embodiment 442. The fusion protein of embodiment 439, wherein the nick site -1, -2, -3, -4, -5, -6, -7, -8, or -9 relative to the 5' end of the PAM sequence.

[2145] Embodiment 443. The fusion protein of embodiment 426, wherein the PEgRNA comprises a guide RNA and at least one nucleic acid extension arm.

[2146] Embodiment 444. The fusion protein of embodiment 443, wherein the extension arm is at the 5′ or the 3′ end of the guide RNA, or at an intramolecular location in the guide RNA.

[2147] Embodiment 445. The fusion protein of embodiment 443, wherein the extension arm comprises (i) a DNA synthesis template sequence comprising a desired nucleotide change, and (ii) a primer binding site.

[2148] Embodiment 446. The fusion protein of embodiment 445, wherein the DNA synthesis template sequence encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to the nick site, wherein the single-strand DNA flap comprises the desired nucleotide change.

[2149] Embodiment 447. The fusion protein of embodiment 443, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12

nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, at least 30 nucleotides, at least 31 nucleotides, at least 32 nucleotides, at least 33 nucleotides, at least 34 nucleotides, at least 35 nucleotides, at least 36 nucleotides, at least 37 nucleotides, at least 38 nucleotides, at least 39 nucleotides, at least 40 nucleotides, at least 41 nucleotides, at least 42 nucleotides, at least 43 nucleotides, at least 44 nucleotides, at least 45 nucleotides, at least 46 nucleotides, at least 47 nucleotides, at least 48 nucleotides, at least 49 nucleotides, or at least 50 nucleotides.

[2150] Embodiment 448. The fusion protein of embodiment 443, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change in the target strand.

[2151] Embodiment 449. The fusion protein of embodiment 443, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5' end.

[2152] Embodiment 450. The fusion protein of embodiment 446, wherein the endogenous DNA sequence having the 5´ end is excised by the cell.

[2153] Embodiment 451. The fusion protein of embodiment 446, wherein the endogenous DNA sequence having the 5' end is excised by a flap endonuclease.

[2154] Embodiment 452. The fusion protein of embodiment 448, wherein cellular repair of the single-strand DNA flap incorporates the desired nucleotide change in the non-target strand, thereby forming a desired product.

[2155] Embodiment 453. The fusion protein of embodiment 449, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence.

[2156] Embodiment 454. The fusion protein of embodiment 449, wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

[2157] Embodiment 455. The fusion protein of embodiment 425, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence to SEQ ID NO: 18.

[2158] Embodiment 456. The fusion protein of embodiment 425, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2159] Embodiment 457. The fusion protein of any one of the previous embodiments, wherein the fusion protein comprises the structure NH2-[napDNAbp]-[domain comprising an RNA-dependent DNA polymerase activity]-COOH; or NH2-[domain comprising an RNA-dependent DNA polymerase activity]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

[2160] Embodiment 458. The fusion protein of embodiment 457, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769 .

[2161] Embodiment 459. The fusion protein of embodiment 425, wherein the desired nucleotide change is a single nucleotide change, an insertion of one or more nucleotides, or a deletion of one or more nucleotides.

[2162] Embodiment 460. The fusion protein of embodiment 459, wherein the insert or deletion is at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22 , at least 23 , at least 24 , at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, or at least 50.

[2163] Embodiment 461. A complex comprising a fusion protein of any of embodiments 425-460 and a PEgRNA, wherein the PEgRNA directs the fusion protein to a target DNA sequence for prime editing.

[2164] Embodiment 462. The complex of embodiment 461, wherein the PEgRNA comprises a guide RNA and a nucleic acid extension arm at the 3′ or 5′ end of the guide RNA or at an intramolecular position in the guide RNA.

[2165] Embodiment 463. The complex of embodiment 462, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to the target DNA sequence.

[2166] Embodiment 464. The complex of embodiment 463, wherein the target DNA sequence comprises a target strand and a complementary non-target strand, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[2167] Embodiment 465. The complex of embodiment 464, wherein the at least one nucleic acid extension arm comprises (i) a DNA synthesis template, and (ii) a primer binding site.

[2168] Embodiment 466. The complex of embodiment 464, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2169] Embodiment 467. The complex of embodiment 465, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[2170] Embodiment 468. The complex of embodiment 465, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[2171] Embodiment 469. The complex of embodiment 461, wherein the napDNAbp is a Cas9 nickase.

[2172] Embodiment 470. The complex of embodiment 461, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[2173] Embodiment 471. The complex of embodiment 461, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2174] Embodiment 472. The complex of embodiment 461, wherein the PEgRNA comprises a guide RNA and a nucleic acid extension arm at the 3´ or 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[2175] Embodiment 473. The complex of embodiment 465, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[2176] Embodiment 474. The complex of embodiment 465, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[2177] Embodiment 475. The complex of embodiment 462, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[2178] Embodiment 476. A polynucleotide encoding the fusion protein of any of embodiments 425-461.

[2179] Embodiment 477. A polynucleotide encoding the PEgRNA of any of the above embodiments.

[2180] Embodiment 478. A vector comprising the polynucleotide of embodiment 476, wherein expression of the fusion protein is under the control of a promoter.

[2181] Embodiment 479. A vector comprising the polynucleotide of embodiment 477, wherein expression of the PEgRNA is under the control of a promoter.

[2182] Embodiment 480. The vector of embodiment 479, wherein the promoter is a U6 promoter.

[2183] Embodiment 481. The vector of embodiment 479, wherein the promoter is a CMV promoter.

[2184] Embodiment 482. The vector of embodiment 480, wherein the PEgRNA is engineered to remove one or more repeating clusters of Ts in the extension arm to improve transcription efficiency by the U6 promoter.

[2185] Embodiment 483. The vector of embodiment 482, wherein the one or more repeating clusters of Ts that is removed comprises at least 3 Ts, at least 4 Ts, at least 5 Ts, at least 6 Ts, at least 7 Ts, at least 8 Ts, at least 9 Ts, at least 10 Ts, at least 11 Ts, at least 12 Ts, at least 13 Ts, at least 14 Ts, at least 15 Ts, at least 16 Ts, at least 17 Ts, at least 18 Ts, at least 19 Ts, or at least 20 Ts.

[2186] Embodiment 484. A cell comprising the fusion protein of any of embodiments 425-460 and an PEgRNA bound to the napDNAbp of the fusion protein.

[2187] Embodiment 485. A cell comprising a complex of any one of embodiments 461-475.

[2188] Embodiment 486. A pharmaceutical composition comprising: (i) a fusion protein of any of embodiments 425-460, the complex of embodiments 461-475, the polynucleotide of embodiments 476-477, or the vector of embodiments 478-483; and (ii) a pharmaceutically acceptable excipient.

[2189] Embodiment 487. A pharmaceutical composition comprising: (i) the complex of embodiments 461-475 (ii) a polymerase provided in trans; and (iii) a pharmaceutically acceptable excipient.

[2190] Embodiment 488. A kit for prime editing comprising: (i) a nucleic acid molecule encoding the fusion protein of any one of embodiments 425-460; and (ii) a nucleic acid molecule encoding a PEgRNA that is capable of directing the fusion protein to a target DNA site, wherein the nucleic acid molecule of (i) and (ii) may be contained within a single DNA construct or separate DNA constructs.

[2191] Embodiment 489. The kit of embodiment 488, wherein the nucleic acid molecule of (i) further comprising a promoter that drives expression of the fusion protein.

[2192] Embodiment 490. The kit of embodiment 488, wherein the nucleic acid molecule of (ii) further comprises a promoter that drives expresssion of the PEgRNA.

[2193] Embodiment 491. The kit of embodiment 490, wherein the promoter is a U6 promoter.

[2194] Embodiment 492. The kit of embodiment 490, wherein the promoter is a CMV promoter.

[2195] Embodiment 493. The fusion protein of embodiment 457, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 174 (1x SGGS), 3888 (2x SGGS), 3889 (3x SGGS), 3890 (1x XTEN), 3891 (1x EAAAK), 3892 (2x EAAAK), and 3893 (3x EAAAK).

GROUP B. PE GUIDES and METHODS OF DESIGN

[2196] Embodiment 1. A PEgRNA comprising a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template.

[2197] Embodiment 2. The PEgRNA of embodiment 1, wherein the nucleic acid extension arm is position at the 3′ or 5′ end of the guide RNA, or at an intramolecular position in the guide RNA, and wherein the nucleic acid extension arm is DNA or RNA.

[2198] Embodiment 3. The PEgRNA of embodiment 1, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[2199] Embodiment 4. The PEgRNA of embodiment 3, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[2200] Embodiment 5. The PEgRNA of embodiment 3, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[2201] Embodiment 6. The PEgRNA of embodiment 1, wherein the at least one nucleic acid extension arm further comprises a primer binding site.

[2202] Embodiment 7. The PEgRNA of embodiment 1, wherein the nucleic acid extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, at least 30 nucleotides, at least 31 nucleotides, at least 32 nucleotides, at least 33 nucleotides, at least 34 nucleotides, at least 35 nucleotides, at least 36 nucleotides, at least 37 nucleotides, at least 38 nucleotides, at least 39 nucleotides, at least 40 nucleotides, at least 41 nucleotides, at least 42 nucleotides, at least 43 nucleotides, at least 44 nucleotides, at least 45 nucleotides, at least 46 nucleotides, at least 47 nucleotides, at least 48 nucleotides, at least 49 nucleotides, or at least 50 nucleotides.

[2203] Embodiment 8. The PEgRNA of embodiment 1, wherein the DNA synthesis template is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[2204] Embodiment 9. The PEgRNA of embodiment 6, wherein the primer binding site is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[2205] Embodiment 10. The PEgRNA of embodiment 1, further comprising at least one additional structure selected from the group consisting of a tRNA, linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[2206] Embodiment 11. The PEgRNA of embodiment 1, wherein the DNA synthesis template encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

[2207] Embodiment 12. The PEgRNA of embodiment 11, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5′ end in the target DNA sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

[2208] Embodiment 13. The PEgRNA of embodiment 11, wherein the endogenous single-stranded DNA having the free 5′ end is excised by the cell.

[2209] Embodiment 14. The PEgRNA of embodiment 13, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[2210] Embodiment 15. The PEgRNA of embodiment 1, comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2211] Embodiment 16. The PEgRNA of embodiment 1, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[2212] Embodiment 17. The PEgRNA of embodiment 6, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[2213] Embodiment 18. The PEgRNA of embodiment 10, wherein the at least one additional structure is located at the 3' or 5' end of the PEgRNA.

[2214] Embodiment 19. The PEgRNA of embodiment 10, wherein the linker comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[2215] Embodiment 20. The PEgRNA of embodiment 10, wherein the stem loop comprises a nucleotide sequence selected from the stem loops described herein.

[2216] Embodiment 21. The PEgRNA of embodiment 10, wherein the hairpin comprises a nucleotide sequence selected from the hairpins described herein.

[2217] Embodiment 22. The PEgRNA of embodiment 10, wherein the toeloop comprises a nucleotide sequence selected from the toeloops described herein.

[2218] Embodiment 23. The PEgRNA of embodiment 10, wherein the aptamer comprises a nucleotide sequence selected from the aptamers described herein.

[2219] Embodiment 24. The PEgRNA of embodiment 10, wherein the RNA-protein recruitment domain comprises a nucleotide sequence selected from the RNA-protein recruitment domain described herein.

[2220] Embodiment 25. A method for designing a PEgRNA for use in prime editing to install a desired nucleotide change in a target nucleotide sequence, wherein said PEgRNA comprises a spacer, gRNA core, and an extension arm, and wherein said extension arm comprises a primer binding site and a DNA synthesis template, said method comprising:

[2221] (i) selecting a desired target edit site in a target nucleotide sequence;

[2222] (ii) obtaining a context nucleotide sequence upstream and downstream from the target edit site;

[2223] (iii) locating putative protospacer adjacent motif (PAM) sites in the context nucleotide sequence which are proximal to the desired target edit site;

[2224] (iv) identifying the corresponding nick sites for each putative PAM site;

[2225] (v) designing the spacer;

[2226] (vi) designing the gRNA core;

[2227] (vii) designing the extension arm; and

[2228] (viii) constructing the full PEgRNA by concatenating the spacer, gRNA core, and the extension arm.

[2229] Embodiment 26. The method of embodiment 25, wherein the step (i) of selecting the desired target edit site comprises selecting a disease-causing mutation.

[2230] Embodiment 27. The method of embodiment 26, wherein the disease-causing mutation is associated with a disease selected from the group consisting of: cancer, autoimmune disorders, neurological disorders, skin disorders, respiratory diseases, and cardiac diseases.

[2231] Embodiment 28. The method of embodiment 25, wherein the step (ii) of obtaining a context nucleotide sequence upstream and downstream from the target edit site comprises obtaining about 50-55 base pairs (bp), about 55-60 bp, about 60-65 bp, about 65-70 bp, about 70-75 bp, about 75-80 bp, about 80-85 bp, about 85-90 bp, about 90-95 bp, about 95-100 bp, about 100-105 bp, about 105-110 bp, about 110-125 bp, about 125-130 bp, about 130-135 bp, about 135-140 bp, about 140-145 bp, about 145-150 bp, about 150-155 bp, about 155-160 bp, about 160-165 bp, about 165-170 bp, about 170-175 bp, about 175-180 bp, about 180-185 bp, about 185-190 bp, about 190-195 bp, about 195-200 bp, about 200-205 bp, about 205-210 bp, about 210-215 bp, about 215-220 bp, about 220-225 bp, about 225-230 bp, about 230-235 bp, about 235-240 bp, about 240-245 bp, or about 245-250 bp of a region that comprises the desired target edit site.

[2232] Embodiment 29. The method of embodiment 28, wherein the desired target edit site is positioned approximately equidistant from each end of the context nucleotide sequence.

[2233] Embodiment 30. The method of embodiment 25, wherein in step (iii), the putative PAM sites are proximal to the desired target edit site.

[2234] Embodiment 31. The method of embodiment 25, wherein in step (iii), the putative PAM sites comprise those with associated nick sites located at a position less than 30 nucleotides from the target edit site, or less than 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 nucleotides from the target edit site.

[2235] Embodiment 32. The method of embodiment 25, wherein in step (iii), the putative PAM sites comprise those with associated nick sites located at a position more than 30 nucleotides from the target edit site, or more than 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 ,85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides from the target edit site.

[2236] Embodiment 33. The method of embodiment 25, wherein in step (iii), the putative PAM sites are associated with one or corresponding napDNAbp which bind to said PAM sites.

[2237] Embodiment 34. The method of embodiment 33, the putative PAM sites and their corresponding napDNAbps are selected from any of the following groupings: (a) SpCas9 of SEQ ID NO: 18-25 and 87-88 and NGG; (b) Sp VQR nCas9; and (c) NGAN.

[2238] Embodiment 35. The method of embodiment 25, wherein the step (v) of designing the spacer comprises determining the complement nucleotide sequence of the protospacer sequence associated with each putative PAM.

[2239] Embodiment 36. The method of embodiment 25, wherein the step (vi) of designing gRNA core comprises in the context of each putative PAM, selecting a gRNA core sequence that is capable of binding to a napDNAbp which is associated with each of said putative PAMs.

[2240] Embodiment 37. The method of embodiment 25, wherein the step (vii) of designing the extension arm comprises designing (a) a DNA synthesis template comprising the edit of interest, and (b) a primer binding site.

[2241] Embodiment 38. The method of embodiment 37, wherein designing the primer binding site comprises (a) identifying a DNA primer on the PAM-containing strand of the target nucleotide sequence, wherein the 3' end of the DNA primer is the first nucleotide upstream of the nick site associated with the PAM site, and (b) designing the complement of the DNA primer, wherein said complement forms the the primer binding site.

[2242] Embodiment 39. The method of embodiment 38, wherein the primer binding site is 8 to 15 nucleotides in length.

[2243] Embodiment 40. The method of embodiment 38, wherein the primer binding site is 12-13 nucleotides if the DNA primer contains about 40-60% GC content.

[2244] Embodiment 41. The method of embodiment 38, wherein the primer binding site is 14-15 nucleotides if the DNA primer contains less than about 40% GC content.

[2245] Embodiment 42. The method of embodiment 38, wherein the primer binding site is 8-11 nucleotides if the DNA primer contains greater than about 60% GC content.

[2246] Embodiment 43. A method of prime editing comprising contacting a target DNA sequence with a PEgRNA of any of embodiments 1-24 and a prime editor fusion protein comprising a napDNAbp and a domain having an RNA-dependent DNA polymerase activity.

[2247] Embodiment 44. The method of embodiment 43, wherein the napDNAbp has a nickase activity.

[2248] Embodiment 45. The method of embodiment 43, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2249] Embodiment 46. The method of embodiment 43, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2250] Embodiment 47. The method of embodiment 43, wherein the napDNAbp is Cas9 nickase (nCas9).

[2251] Embodiment 48. The method of embodiment 43, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2252] Embodiment 49. The method of embodiment 43, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2253] Embodiment 50. The method of embodiment 43, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2254] Embodiment 51. The method of embodiment 43, wherein the domain comprising an RNA-dependent DNA polymerase activity is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

GROUP C. PE COMPLEXES

[2255] Embodiment 1. A complex for prime editing comprising:

[2256] (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a domain comprising an RNA-dependent DNA polymerase activity; and

[2257] (ii) a prime editing guide RNA (PEgRNA).

[2258] Embodiment 2. The complex of embodiment 1, wherein the fusion protein is capable of carrying out prime editing in the presence of the prime editing guide RNA (PEgRNA) to install a desired nucleotide change in a target sequence.

[2259] Embodiment 3. The complex of embodiment 11, wherein the napDNAbp has a nickase activity.

[2260] Embodiment 4. The complex of embodiment 1, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2261] Embodiment 5. The complex of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2262] Embodiment 6. The complex of embodiment 1, wherein the napDNAbp is Cas9 nickase (nCas9).

[2263] Embodiment 7. The complex of embodiment 1, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2264] Embodiment 8. The complex of embodiment 1, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2265] Embodiment 9. The complex of embodiment 1, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2266] Embodiment 10. The complex of embodiment 1, wherein the domain comprising an RNA-dependent DNA polymerase activity is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

[2267] Embodiment 11. The complex of embodiment 1, wherein the fusion protein when complexed with a PEgRNA is capable of binding to a target DNA sequence.

[2268] Embodiment 12. The complex of embodiment 1, wherein the PEgRNA comprises a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template.

[2269] Embodiment 13. The complex of embodiment 12, wherein the nucleic acid extension arm is position at the 3′ or 5′ end of the guide RNA, or at an intramolecular position in the guide RNA, and wherein the nucleic acid extension arm is DNA or RNA.

[2270] Embodiment 14. The complex of embodiment 12, wherein the PEgRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

[2271] Embodiment 15. The complex of embodiment 14, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[2272] Embodiment 16. The complex of embodiment 12, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

[2273] Embodiment 17. The complex of embodiment 12, wherein the at least one nucleic acid extension arm further comprises a primer binding site.

[2274] Embodiment 18. The complex of embodiment 12, wherein the nucleic acid extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, at least 30 nucleotides, at least 31 nucleotides, at least 32 nucleotides, at least 33 nucleotides, at least 34 nucleotides, at least 35 nucleotides, at least 36 nucleotides, at least 37 nucleotides, at least 38 nucleotides, at least 39 nucleotides, at least 40 nucleotides, at least 41 nucleotides, at least 42 nucleotides, at least 43 nucleotides, at least 44 nucleotides, at least 45 nucleotides, at least 46 nucleotides, at least 47 nucleotides, at least 48 nucleotides, at least 49 nucleotides, or at least 50 nucleotides.

[2275] Embodiment 19. The complex of embodiment 12, wherein the DNA synthesis template is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[2276] Embodiment 20. The complex of embodiment 17, wherein the primer binding site is at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, or at least 15 nucleotides in length.

[2277] Embodiment 21. The complex of embodiment 12, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[2278] Embodiment 22. The complex of embodiment 12, wherein the DNA synthesis template encodes a single-strand DNA flap that is complementary to an endogenous DNA sequence adjacent to a nick site, wherein the single-strand DNA flap comprises a desired nucleotide change.

[2279] Embodiment 23. The complex of embodiment 22, wherein the single-stranded DNA flap displaces an endogenous single-strand DNA having a 5′ end in the target DNA

sequence that has been nicked, and wherein the endogenous single-strand DNA is immediately adjacent downstream of the nick site.

[2280] Embodiment 24. The complex of embodiment 23, wherein the endogenous single-stranded DNA having the free 5´ end is excised by the cell.

[2281] Embodiment 25. The complex of embodiment 23, wherein cellular repair of the single-strand DNA flap results in installation of the desired nucleotide change, thereby forming a desired product.

[2282] Embodiment 26. The complex of embodiment 12, wherein the PEgRNA comprises the nucleotide sequence of SEQ ID NOs: 18-36, or a nucleotide sequence having at least 85%, or at least 90%, or at least 95%, or at least 98%, or at least 99% sequence identity with any one of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2283] Embodiment 27. The complex of embodiment 12, wherein the DNA synthesis template comprises a nucleotide sequence that is at least 80%, or 85%, or 90%, or 95%, or 99% identical to the endogenous DNA target.

[2284] Embodiment 28. The complex of embodiment 17, wherein the primer binding site hybridizes with a free 3' end of the cut DNA.

[2285] Embodiment 29. The complex of embodiment 21, wherein the at least one additional structure is located at the 3' or 5' end of the PEgRNA.

[2286] Embodiment 30. The complex of embodiment 29, wherein the linker comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[2287] Embodiment 31. The complex of embodiment 29, wherein the stem loop comprises a nucleotide sequence selected from the stem loops described herein.

[2288] Embodiment 32. The complex of embodiment 29, wherein the hairpin comprises a nucleotide sequence selected from the hairpins described herein.

[2289] Embodiment 33. The complex of embodiment 29, wherein the toeloop comprises a nucleotide sequence selected from the toeloops described herein.

[2290] Embodiment 34. The complex of embodiment 29, wherein the aptamer comprises a nucleotide sequence selected from the aptamers described herein.

[2291] Embodiment 35. The complex of embodiment 29, wherein the RNA-protein recruitment domain comprises a nucleotide sequence selected from the RNA-protein recruitment domains described herein.

[2292] Embodiment 36. The complex of embodiment 1, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

[2293] Embodiment 37. The complex of embodiment 36, wherein the R-loop comprises (i) an RNA-DNA hybrid comprising the PEgRNA and the target strand, and (ii) the complementary non-target strand.

[2294] Embodiment 38. The complex of embodiment 37, wherein the target or the complementary non-target strand is nicked to form a priming sequence having a free 3´ end.

[2295] Embodiment 39. The complex of embodiment 38, wherein the nick site is upstream of the PAM sequence on the target strand.

[2296] Embodiment 40. The complex of embodiment 38, wherein the nick site is upstream of the PAM sequence on the non-target strand.

[2297] Embodiment 41. The complex of embodiment 38, wherein the nick site -1, -2, -3, -4, -5, -6, -7, -8, or -9 relative to the 5' end of the PAM sequence.

[2298] Embodiment 42. The complex of embodiment 22, wherein the single-strand DNA flap hybridizes to the endogenous DNA sequence adjacent to the nick site, thereby installing the desired nucleotide change in the target strand.

[2299] Embodiment 43. The complex of embodiment 22, wherein the single-stranded DNA flap displaces the endogenous DNA sequence adjacent to the nick site and which has a free 5' end.

[2300] Embodiment 44. The complex of embodiment 22, wherein the endogenous DNA sequence having the 5´ end is excised by the cell.

[2301] Embodiment 45. The complex of embodiment 44, wherein the endogenous DNA sequence having the 5' end is excised by a flap endonuclease.

[2302] Embodiment 46. The complex of embodiment 43, wherein cellular repair of the single-strand DNA flap incorporates the desired nucleotide change in the non-target strand, thereby forming a desired product.

[2303] Embodiment 47. The complex of embodiment 46, wherein the desired nucleotide change is installed in an editing window that is between about -4 to +10 of the PAM sequence, or between about -10 to +20 of the PAM sequence, or between about -20 to +40 of the PAM sequence, or between about -30 to +100 of the PAM sequence.

[2304] Embodiment 48. The complex of embodiment 47, wherein the desired nucleotide change is installed at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 nucleotides downstream of the nick site.

[2305] Embodiment 49. The complex of any one of the previous embodiments, wherein the fusion protein comprises the structure NH2-[napDNAbp]-[domain comprising an RNA-dependent DNA polymerase activity]-COOH; or NH2-[domain comprising an RNA-dependent DNA polymerase activity]-[napDNAbp]-COOH, wherein each instance of "]-[" indicates the presence of an optional linker sequence.

[2306] Embodiment 50. The complex of embodiment 49, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs: 127, 165-176, 446, 453, and 767-769.

[2307] Embodiment 51. The complex of embodiment 1, wherein the fusion protein further comprises a linker that joins the napDNAbp and the domain comprising an RNA-dependent DNA polymerase activity.

[2308] Embodiment 52. complex of embodiment 51, wherein the linker sequence comprises an amino acid sequence of SEQ ID NOs. 3887 (1x SGGS), 3888 (2x SGGS), 3889 (3x SGGS), 3890 (1x XTEN), 3891 (1x EAAAK), 3892 (2x EAAAK), and 3893 (3x EAAAK).

GROUP D. PE METHOD FOR CORRECTING MUTATIONS

[2309] Embodiment 1. A method for installing a desired nucleotide change in a double-stranded DNA sequence, the method comprising: contacting the double-stranded DNA sequence with a complex comprising a fusion protein and a PEgRNA, wherein the fusion protein comprises a napDNAbp and a polymerase, and wherein the PEgRNA comprises a DNA synthesis template comprising the desired nucleotide change and a primer binding site;

[2310] thereby nicking the double-stranded DNA sequence, thereby generating a free single-strand DNA having a 3′ end;

[2311] thereby hybridizing the 3′ end of the free single-strand DNA to the primer binding site, thereby priming the polymerase;

[2312] thereby polymerizing a strand of DNA from the 3´ end hybridized to the primer binding site, thereby generating a single-strand DNA flap comprising the desired nucleotide change and which is complementary to the DNA synthesis template;

[2313] thereby replacing an endogenous DNA strand adjacent the cut site with the single-strand DNA flap, thereby installing the desired nucleotide change in the double-stranded DNA sequence.

[2314] Embodiment 2. The method of embodiment 1, wherein replacing an endogenous DNA strand comprises: (i) hybridizing the single-strand DNA flap to the endogenous DNA strand adjacent the cut site to create a sequence mismatch; (ii) excising the endogenous DNA strand; and (iii) repairing the mismatch to form the desired product comprising the desired nucleotide change in both strands of DNA.

[2315] Embodiment 3. The method of embodiment 1, wherein the desired nucleotide change is a single nucleotide substitution, a deletion, or an insertion.

[2316] Embodiment 4. The method of embodiment 3, wherein the single nucleotide substitution is a transition or a transversion.

[2317] Embodiment 5. The method of embodiment 1, wherein the desired nucleotide change is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[2318] Embodiment 6. The method of embodiment 1, wherein the desired nucleotide change converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2319] Embodiment 7. The method of embodiment 1, wherein the desired nucleotide change is an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[2320] Embodiment 8. The method of embodiment 1, wherein the desired nucleotide change corrects a disease-associated gene.

[2321] Embodiment 9. The method of embodiment 8, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

[2322] Embodiment 10. The method of embodiment 8, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[2323] Embodiment 11. The method of embodiment 1, wherein the napDNAbp is a nuclease dead Cas9 (dCas9), a Cas9 nickase (nCas9), or a nuclease active Cas9.

[2324] Embodiment 12. The method of embodiment 1, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[2325] Embodiment 13. The method of embodiment 1, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2326] Embodiment 14. The method of embodiment 1, wherein the polymerase is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739- 741, and 766.

[2327] Embodiment 15. The method of embodiment 1, wherein the polymerase is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739- 741, and 766.

[2328] Embodiment 16. The method of embodiment 1, wherein the PEgRNA comprises a nucleic acid extension arm at the 3` or 5` ends or at an intramolecular location in the guide RNA, wherein the extension arm comprises the DNA synthesis template sequence and the primer binding site.

[2329] Embodiment 17. The method of embodiment 16, wherein the extension arm is at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, or at least 25 nucleotides in length.

[2330] Embodiment 18. The method of embodiment 1, wherein the PEgRNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2331] Embodiment 19. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus, comprising: contacting the DNA molecule with a nucleic acid programmable DNA binding protein (napDNAbp) and a PEgRNA which targets the napDNAbp to the target locus, wherein the PEgRNA comprises a reverse transcriptase (RT) template sequence comprising at least one desired nucleotide change and a primer binding site;

[2332] thereby forming an exposed 3′ end in a DNA strand at the target locus;

[2333] thereby hybridizing the exposed 3′ end to the primer binding site to prime reverse transcription;

[2334] thereby synthesizing a single strand DNA flap comprising the at least one desired nucleotide change based on the RT template sequence by reverse transcriptase;

[2335] thereby incorporating the at least one desired nucleotide change into the corresponding endogenous DNA, thereby introducing one or more changes in the nucleotide sequence of the DNA molecule at the target locus.

[2336] Embodiment 20. The method of embodiment 19, wherein the one or more changes in the nucleotide sequence comprises a transition.

[2337] Embodiment 21. The method of embodiment 19, wherein the transition is selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[2338] Embodiment 22. The method of embodiment 19, wherein the one or more changes in the nucleotide sequence comprises a transversion.

[2339] Embodiment 23. The method of embodiment 22, wherein the transversion is selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[2340] Embodiment 24. The method of embodiment 19, wherein the one or more changes in the nucleotide sequence comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (9) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2341] Embodiment 25. The method of embodiment 19, wherein the one or more changes in the nucleotide sequence comprises an insertion or deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides.

[2342] Embodiment 26. The method of embodiment 19, wherein the one or more changes in the nucleotide sequence comprises a correction to a disease-associated gene.

[2343] Embodiment 27. The method of embodiment 26, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

[2344] Embodiment 28. The method of embodiment 26, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[2345] Embodiment 29. The method of embodiment 19, wherein the napDNAbp is a nuclease active Cas9 or variant thereof.

[2346] Embodiment 30. The method of embodiment 19, wherein the napDNAbp is a nuclease inactive Cas9 (dCas9) or Cas9 nickase (nCas9), or a variant thereof.

[2347] Embodiment 31. The method of embodiment 19, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[2348] Embodiment 32. The method of embodiment 19, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2349] Embodiment 33. The method of embodiment 19, wherein the reverse transcriptase is introduced in trans.

[2350] Embodiment 34. The method of embodiment 19, wherein the napDNAbp comprises a fusion to a reverse transcriptase.

[2351] Embodiment 35. The method of embodiment 19, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2352] Embodiment 36. The method of embodiment 19, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2353] Embodiment 37. The method of embodiment 19, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises nicking the DNA strand with a nuclease.

[2354] Embodiment 38. The method of embodiment 37, wherein the nuclease is the napDNAbp, is provided as a fusion domain of napDNAbp, or is provided in trans.

[2355] Embodiment 39. The method of embodiment 19, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises contacting the DNA strand with a chemical agent.

[2356] Embodiment 40. The method of embodiment 19, wherein the step of forming an exposed 3' end in the DNA strand at the target locus comprises introducing a replication error.

[2357] Embodiment 41. The method of embodiment 19, wherein the step of contacting the DNA molecule with the napDNAbp and the guide RNA forms an R-loop.

[2358] Embodiment 42. The method of embodiment 41, wherein the DNA strand in which the exposed 3' end is formed is in the R-loop.

[2359] Embodiment 43. The method of embodiment 19, wherein the PEgRNA comprises an extension arm that comprises the reverse transcriptase (RT) template sequence and the primer binding site.

[2360] Embodiment 44. The method of embodiment 43, wherein the extension arm is at the 3´ end of the guide RNA, the 5´ end of the guide RNA, or at an intramolecular position in the guide RNA.

[2361] Embodiment 45. The method of embodiment 19, wherein the PEgRNA further comprises at least one additional structure selected from the group consisting of a linker, a stem loop, a hairpin, a toeloop, an aptamer, or an RNA-protein recruitment domain.

[2362] Embodiment 46. The method of embodiment 19, wherein the PEgRNA further comprises a homology arm.

[2363] Embodiment 47. The method of embodiment 19, wherein the RT template sequence is homologous to the corresponding endogenous DNA.

[2364] Embodiment 48. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change;

[2365] thereby conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change;

[2366] thereby incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2367] Embodiment 49. The method of embodiment 48, wherein the RT template is located at the 3´ end of the guide RNA, the 5' end of the guide RNA, or at an intramolecular location in the guide RNA.

[2368] Embodiment 50. The method of embodiment 48, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

[2369] Embodiment 51. The method of embodiment 48, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[2370] Embodiment 52. The method of embodiment 48, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[2371] Embodiment 53. The method of embodiment 48, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2372] Embodiment 54. A method for replacing a trinucleotide repeat expansion mutation in a target DNA molecule with a healthy sequence comprising a healthy number of repeat trinucleotides, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising DNA synthesis template comprising the replacement sequence and a primer binding site; (b) conducting prime editingto generate a single strand DNA comprising the replacement sequence; and (c) incorporating the single strand DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2373] Embodiment 55. The method of embodiment 54, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2374] Embodiment 56. The method of embodiment 54, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2375] Embodiment 57. The method of embodiment 54, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2376] Embodiment 58. The method of embodiment 54, wherein the guide RNA comprises SEQ ID NOs: 222.

[2377] Embodiment 59. The method of embodiment 54, wherein the step of (b) conducting prime editing comprises generating a 3' end primer binding sequence at the target locus that is capable of priming polymerase by annealing to the primer binding site on the guide RNA.

[2378] Embodiment 60. The method of embodiment 54, wherein the trinucleotide repeat expansion mutation is associated with Huntington's Disease, Fragile X syndrome, or Friedreich's ataxia.

[2379] Embodiment 61. The method of embodiment 54, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of CAG triplets.

[2380] Embodiment 62. The method of embodiment 54, wherein the trinucleotide repeat expansion mutation comprises a repeating unit of GAA triplets.

[2381] Embodiment 63. A method for introducing one or more changes in the nucleotide sequence of a DNA molecule at a target locus by target-primed reverse transcription, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase and (ii) a guide RNA comprising an RT template comprising a desired nucleotide change;

[2382] thereby conducting target-primed reverse transcription of the RT template to generate a single strand DNA comprising the desired nucleotide change;

[2383] thereby incorporating the desired nucleotide change into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2384] Embodiment 64. The method of embodiment 63, wherein the RT template is located at the 3´ end of the guide RNA, the 5' end of the guide RNA, or at an intramolecular location in the guide RNA.

[2385] Embodiment 65. The method of embodiment 63, wherein the desired nucleotide change comprises a transition, a transversion, an insertion, or a deletion, or any combination thereof.

[2386] Embodiment 66. The method of embodiment 63, wherein the desired nucleotide change comprises a transition selected from the group consisting of: (a) T to C; (b) A to G; (c) C to T; and (d) G to A.

[2387] Embodiment 67. The method of embodiment 63, wherein the desired nucleotide change comprises a transversion selected from the group consisting of: (a) T to A; (b) T to G; (c) C to G; (d) C to A; (e) A to T; (f) A to C; (g) G to C; and (h) G to T.

[2388] Embodiment 68. The method of embodiment 63, wherein the desired nucleotide change comprises changing (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a

G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2389] Embodiment 69. A method of preventing or halting the progression of a prion disease by installing on or more protective mutations into PRNP encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety;

[2390] thereby polymerizing a single strand DNA sequence encoding the protective mutation;

[2391] thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

[2392] wherein the method produces a recombinant target nucleotide sequence that encodes a PRNP comprising a protective mutation and which is resistant to misfolding.

[2393] Embodiment 70. The method of embodiment 69, wherein the prion disease is a human prion disease.

[2394] Embodiment 71. The method of embodiment 69, wherein the prion disease is an animal prion disease.

[2395] Embodiment 72. The method of embodiment 69, wherein the prion disease is Creutzfeldt-Jakob Disease (CJD), Variant Creutzfeldt-Jakob Disease (vCJD), Gerstmann-Straussler-Scheinker Syndrome, Fatal Familial Insomnia, or Kuru.

[2396] Embodiment 73. The method of embodiment 69, wherein the prion disease is Bovine Spongiform Encephalopathy (BSE or “mad cow disease”), Chronic Wasting Disease (CWD), Scrapie, Transmissible Mink Encephalopathy, Feline Spongiform Encephalopathy, and Ungulate Spongiform Encephalopathy.

[2397] Embodiment 74. The method of embodiment 69, wherein the wildtype PRNP amino acid sequence is SEQ ID NOs: 291-292.

[2398] Embodiment 75. The method of embodiment 69, wherein the method results in a modified PRNP amino acid sequence selected from the group consisting of SEQ ID NOs: 293-309, 311-323, wherein said modified PRNP protein is resistant to misfolding.

[2399] Embodiment 76. The method of embodiment 69, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2400] Embodiment 77. The method of embodiment 69, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2401] Embodiment 78. The method of embodiment 69, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2402] Embodiment 79. The method of embodiment 69, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2403] Embodiment 80. A method of treating CDKL5 Deficiency Disorder by correcting a mutation in the cyclin-dependent kinase-like 5 gene (CDKL5) in a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template that corrects the mutation in CDKL5;

[2404] thereby polymerizing a single strand DNA sequence encoding the edit;

[2405] thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

[2406] wherein the method produces a recombinant target nucleotide sequence that encodes a repaired CDKL5 gene.

[2407] Embodiment 81. The method of embodiment D80, wherein the mutation in CDKL5 is 1412delA.

GROUP E. PE METHODS FOR MODIFYING PROTEIN STRUCTURE/FUNCTION AND/OR MUTAGENESIS

[2408] Embodiment 1. A method for mutagenizing a DNA molecule at a target locus by prime editing, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone polymer (e.g., error-prone reverse transcriptase), and (ii) a guide RNA comprising an edit template comprising a desired nucleotide change; thereby polymerizing a single stranded DNA templated from the edit template; and incorporating the

single stranded DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

[2409] Embodiment 2. The method of any prior embodiment, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2410] Embodiment 3. The method of any prior embodiment, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2411] Embodiment 4. The method of embodiment 1, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2412] Embodiment 5. The method of embodiment 1, wherein the guide RNA comprises SEQ ID NOs: 222.

[2413] Embodiment 6. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety;

[2414] thereby polymerizing a single strand DNA sequence encoding the immunoepitope;

[2415] thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

[2416] wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

[2417] Embodiment 7. The method of embodiment 6, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); and neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

[2418] Embodiment 8. The method of embodiment 6, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2419] Embodiment 9. The method of embodiment 6, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2420] Embodiment 10. The method of embodiment 6, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2421] Embodiment 11. The method of embodiment 6, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2422] Embodiment 12. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase, and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain;

[2423] thereby polymerizing a single strand DNA sequence encoding the immunoepitope;

[2424] thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

[2425] wherein the method produces a modified target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the small molecule dimerization domain.

[2426] Embodiment 13. The method of embodiment 12 further comprising conducting the method on a second protein of interest.

[2427] Embodiment 14. The method of embodiment 13, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

[2428] Embodiment 15. The method of embodiment 12, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

[2429] Embodiment 16. The method of embodiment 12, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

[2430] Embodiment 17. The method of embodiment 12, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 490 and 493-494.

[2431] Embodiment 18. The method of embodiment 12, wherein the small molecule is a dimer of a small molecule as described herein.

[2432] Embodiment 19. The method of embodiment 12, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2433] Embodiment 20. The method of embodiment 12, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2434] Embodiment 21. The method of embodiment 12, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2435] Embodiment 22. The method of embodiment 12, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2436] Embodiment 23. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

[2437] Embodiment 24. The method of embodiment 23, wherein the peptide tag is used for purification and/or detection of the protein.

[2438] Embodiment 25. The method of embodiment 23, wherein the peptide tag is a poly-histidine (e.g., HHHHHH) (SEQ ID NO: 252-262), FLAG (e.g., DYKDDDDK) (SEQ ID NO: 2), V5 (e.g., GKPIPNPLLGLDST) (SEQ ID NO: 3), GCN4, HA (e.g., YPYDVPDYA) (SEQ ID NO: 5), Myc (e.g. EQKLISEED) (SEQ ID NO: 6), or GST.

[2439] Embodiment 26. The method of embodiment 23, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 1-6, 245-249, 252-262, 264-273, 275-276, 281, 278-288, and 622.

[2440] Embodiment 27. The method of embodiment 23, wherein the peptide tag is fused to the protein by a linker.

[2441] Embodiment 28. The method of embodiment 23, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

[2442] Embodiment 29. The method of embodiment 23, wherein the linker has an amino acid sequence of SEQ ID NO: 127, 165-176, 446, 453, and 767-769.

[2443] Embodiment 30. The method of embodiment 23, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2444] Embodiment 31. The method of embodiment 23, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[2445] Embodiment 32. The method of embodiment 23, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

[2446] Embodiment 33. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

[2447] Embodiment 34. The method of embodiment 33, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, or SUMOylation state of the protein of interest.

GROUP F. PE DELIVERY METHODS AND COMPOSITIONS

[2448] Embodiment 1. A polypeptide comprising an N-terminal half or a C-terminal half of a prime editor fusion protein.

[2449] Embodiment 2. The polypeptide of embodiment 1, wherein the prime editor fusion protein comprises a nucleic acid programmable DNA binding protein (napDNAbp) domain and a polymerase domain.

[2450] Embodiment 3. The polypeptide of embodiment 1, wherein the prime editor fusion protein is capable of carrying out prime editing in the presence of a prime editing guide RNA (PEgRNA).

[2451] Embodiment 4. The polypeptide of embodiment 2, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2452] Embodiment 5. The polypeptide of embodiment 2, wherein the napDNAbp is a nuclease with nickase activity.

[2453] Embodiment 6. The polypeptide of embodiment 2, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2454] Embodiment 7. The polypeptide of embodiment 2, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2455] Embodiment 8. The polypeptide of embodiment 1, wherein the polypeptide is formed by splitting a prime editor fusion protein at a split site.

[2456] Embodiment 9. The polypeptide of embodiment 8, wherein the split site is a peptide bond in the napDNAbp domain.

[2457] Embodiment 10. The polypeptide of embodiment 8, wherein the split site is a peptide bond in the polymerase domain.

[2458] Embodiment 11. The polypeptide of embodiment 8, wherein the split site is a peptide bond in a linker between the napDNAbp domain and the polymerase domain.

[2459] Embodiment 12. The polypeptide of embodiment 9, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250, 250-300, 300-350,

350-400, 400-450, 450-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of SEQ ID NO: 18 (canonical SpCas9), or between any two equivalent amino acid residues of an SpCas9 homolog or equivalent of SEQ ID NO: 18.

[2460] Embodiment 13. The polypeptide of embodiment 9, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-600, or 600-667 of SEQ ID NO: 89 (canonical reverse transcriptase, M-MLV RT), or between any two equivalent amino acid residues of a reverse transcriptase homolog or equivalent of SEQ ID NO: 89.

[2461] Embodiment 14. The polypeptide of embodiment 1, wherein the polypeptide is an N-terminal half of a prime editor fusion protein.

[2462] Embodiment 15. The polypeptide of embodiment 1, wherein the polypeptide is a C-terminal half of a prime editor fusion protein.

[2463] Embodiment 16. A nucleotide sequence encoding a polypeptide of any of embodiments 1-15 and optionally a PEgRNA.

[2464] Embodiment 17. A virus genome comprising a nucleotide sequence encoding a polypeptide of any of embodiments 1-15 and optionally a PEgRNA.

[2465] Embodiment 18. The virus genome of embodiment 17, wherein the nucleotide sequence further comprises a promoter sequence suitable for expressing the polypeptide of any of embodiments 1-15.

[2466] Embodiment 19. The virus genome of embodiment 17, wherein the nucleotide sequence further comprises a sequence encoding a PEgRNA.

[2467] Embodiment 20. A virus particle comprising a genome comprising a nucleotide sequence encoding a polypeptide of any of embodiments 1-15 and optionally a PEgRNA.

[2468] Embodiment 21. The virus particle of embodiment 20, wherein the virus particle is an adenovirus particle, an adeno-associated virus particle, or a lentivirus particle.

[2469] Embodiment 22. The virus particle of embodiment 20, wherein the polypeptide encoded by the genome is an N terminal half of a prime editor fusion protein.

[2470] Embodiment 23. The virus particle of embodiment 20, wherein the polypeptide encoded by the genome is a C terminal half of a prime editor fusion protein.

[2471] Embodiment 24. A pharmaceutical composition comprising a virus particle of any of embodiments 20-23 and a pharmaceutical excipient.

[2472] Embodiment 25. A pharmaceutical composition comprising a virus particle of embodiment 22 (encoding the N terminal half) and a pharmaceutical excipient.

[2473] Embodiment 26. A pharmaceutical composition comprising a virus particle of embodiment 23 (encoding the C terminal half) and a pharmaceutical excipient.

[2474] Embodiment 27. A ribonucleoprotein (RNP) complex comprising a nucleotide sequence encoding a polypeptide of any of embodiments 1-15 and optionally a PEgRNA.

[2475] Embodiment 28. The ribonucleoprotein (RNP) complex of embodiment 27, wherein the polypeptide encoded by the genome is an N-terminal half of a prime editor fusion protein.

[2476] Embodiment 29. The ribonucleoprotein (RNP) complex of embodiment 27, wherein the polypeptide encoded by the genome is a C-terminal half of a prime editor fusion protein.

[2477] Embodiment 30. A pharmaceutical composition comprising a ribonucleoprotein (RNP) complex of any of embodiments 27-29 and a pharmaceutical excipient.

[2478] Embodiment 31. A pharmaceutical composition comprising the ribonucleoprotein (RNP) complex of embodiment 28 (encoding the N-terminal half) and a pharmaceutical excipient.

[2479] Embodiment 32. A pharmaceutical composition comprising the ribonucleoprotein (RNP) complex of embodiment 29 (encoding the C-terminal half) and a pharmaceutical excipient.

[2480] Embodiment 33. A pharmaceutical composition comprising a first AAV particle and a second AAV particle, wherein the first AAV vector expresses an N-terminal half of a prime editor fusion protein and the second AAV vector expresses a C-terminal half of a prime editor fusion protein, wherein the N-terminal half and the C-terminal half are combined within the cell to reconstitute the prime editor.

[2481] Embodiment 34. The pharmaceutical composition of embodiment 33, wherein the first or second AAV particle also expresses a PEgRNA that targets the reconstituted prime editor to a target DNA site.

[2482] Embodiment 35. The pharmaceutical composition of embodiment 33, wherein the prime editor fusion protein comprises a nucleic acid programmable DNA binding protein (napDNAbp) domain and a polymerase domain.

[2483] Embodiment 36. The pharmaceutical composition of embodiment 33, wherein the prime editor fusion protein is capable of carrying out prime editing in the presence of a prime editing guide RNA (PEgRNA).

[2484] Embodiment 37. The pharmaceutical composition of embodiment 35, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2485] Embodiment 38. The pharmaceutical composition of embodiment 35, wherein the napDNAbp is a nuclease with a nickase activity.

[2486] Embodiment 39. The pharmaceutical composition of embodiment 35, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2487] Embodiment 40. The pharmaceutical composition of embodiment 35, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2488] Embodiment 41. The pharmaceutical composition of embodiment 33, wherein N-terminal and C-terminal halves are formed by splitting the prime editor fusion protein at a split site.

[2489] Embodiment 42. The pharmaceutical composition of embodiment 41, wherein the split site is a peptide bond in a napDNAbp domain.

[2490] Embodiment 43. The pharmaceutical composition of embodiment 41, wherein the split site is a peptide bond in a polymerase domain.

[2491] Embodiment 44. The pharmaceutical composition of embodiment 41, wherein the split site is a peptide bond in a linker.

[2492] Embodiment 45. The pharmaceutical composition of embodiment 41, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250,

250-300, 300-350, 350-400, 400-450, 450-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of SEQ ID NO: 18 (canonical SpCas9), or between any two equivalent amino acid residues of an SpCas9 homolog or equivalent of SEQ ID NO: 18.

[2493] Embodiment 46. The pharmaceutical composition of embodiment 41, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-600, or 600-667 of SEQ ID NO: 89 (canonical reverse transcriptase, M-MLV RT), or between any two equivalent amino acid residues of a reverse transcriptase homolog or equivalent of SEQ ID NO: 89.

[2494] Embodiment 47. The pharmaceutical composition of embodiment 33, wherein the N-terminal half of the prime editor fusion protein has an amino acid sequence encoding the N-terminal prime editor fusion proteins as described herein.

[2495] Embodiment 48. The pharmaceutical composition of embodiment 33, wherein the C-terminal half of the prime editor fusion protein has an amino acid sequence encoding the N-terminal prime editor fusion proteins as described herein.

[2496] Embodiment 49. A method of delivering a prime editor fusion protein to a cell comprising transfecting the cell with a first AAV particle and a second AAV particle, wherein the first AAV vector expresses an N-terminal half of a prime editor fusion protein and the second AAV vector expresses a C-terminal half of a prime editor fusion protein, wherein the N-terminal half and the C-terminal half are combined within the cell to reconstitute the prime editor fusion protein.

[2497] Embodiment 50. The method of embodiment 49, wherein the first or second AAV particle also expresses a PEgRNA that targets the reconstituted prime editor to a target DNA site.

[2498] Embodiment 51. The method of embodiment 49, wherein the prime editor fusion protein comprises a nucleic acid programmable DNA binding protein (napDNAbp) domain and a polymerase domain.

[2499] Embodiment 52. The method of embodiment 49, wherein the prime editor fusion protein is capable of carrying out prime editing in the presence of an prime editing guide RNA (PEgRNA).

[2500] Embodiment 53. The method of embodiment 51, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2501] Embodiment 54. The method of embodiment 53, wherein the napDNAbp is a nuclease with nickase activity.

[2502] Embodiment 55. The method of embodiment 53, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2503] Embodiment 56. The method of embodiment 53, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute and optionally has a nickase activity.

[2504] Embodiment 57. The method of embodiment 49, wherein N-terminal and C-terminal halves are formed by splitting the prime editor fusion protein at a split site.

[2505] Embodiment 58. The method of embodiment 57, wherein the split site is a peptide bond in a napDNAbp domain.

[2506] Embodiment 59. The method of embodiment 57, wherein the split site is a peptide bond in a polymerase domain.

[2507] Embodiment 60. The method of embodiment 57, wherein the split site is a peptide bond in a linker.

[2508] Embodiment 61. The method of embodiment 57, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8 and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-600, 600-700, 700-800, 800-900, 900-1000, 1000-1100, 1100-1200, 1200-1300, or 1300-1368 of SEQ ID NO: 18 (canonical SpCas9), or between any two equivalent amino acid residues of an SpCas9 homolog or equivalent of SEQ ID NO: 18.

[2509] Embodiment 62. The method of embodiment 57, wherein the split site is in the peptide bond between residues 1 and 2, 2 and 3, 3 and 4, 4 and 5, 5 and 6, 6 and 7, 7 and 8, 8

and 9, 9 and 10, 10 and 11, 11 and 12, 12 and 13, 13 and 14, 14 and 15, 16 and 17, 17 and 18, 18 and 19, 19 and 20, 20 and 21, 21 and 22, 22 and 23, 23 and 24, 24 and 25, 25 and 26, 26 and 27, 27 ad 28, 28 and 29, 29 and 30, 30 and 31, 31 and 32, 32 and 33, 33 and 34, 34 and 35, 35 and 36, 36 and 37, 37 and 38, 38 and 39, 39 and 40, 40 and 41, 41 and 42, 42 and 43, 43 and 44, 44 and 45, 45 and 46, 46 and 47, 47 and 48, 48 and 49, 49 and 50, or between any two residues between residues 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-600, or 600-667 of SEQ ID NO: 89 (canonical reverse transcriptase, M-MLV RT), or between any two equivalent amino acid residues of a reverse transcriptase homolog or equivalent of SEQ ID NO: 89.

[2510] Embodiment 63. The method of embodiment 49, wherein the N-terminal half of the prime editor fusion protein has an amino acid sequence encoding the N-terminal prime editor fusion proteins as described herein.

[2511] Embodiment 64. The method of embodiment 49, wherein the C-terminal half of the prime editor fusion protein has an amino acid sequence encoding the C-terminal prime editor fusion proteins as described herein.

[2512] Embodiment 65. The method of embodiment 49, wherein the first AAV particle comprises a recombinant AAV genome comprising a nucleotide sequence which encodes the first prime editor component.

[2513] Embodiment 66. The method of embodiment 49, wherein the second AAV particle comprises a recombinant AAV genome comprising a nucleotide sequence which encodes the second prime editor component.

[2514] Embodiment 67. The method of embodiment 49, wherein the transfecting step is conducted in vivo.

[2515] Embodiment 68. The method of embodiment 49, wherein the transfecting step is conducted ex vivo.

[2516] Embodiment 69. The method of embodiment 50, wherein the target DNA site is a disease-associated gene.

[2517] Embodiment 70. The method of embodiment 69, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease; Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease;

Smith-Lemli-Opitz Syndrome; a trinucleotide repeat disorder; a prion disease; and Tay-Sachs Disease.

[2518] Embodiment 71. The method of embodiment 69, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[2519] Embodiment 72. The method of embodiment 51, wherein the programmable DNA binding protein (napDNAbp) domain.

[2520] Embodiment 73. The method of embodiment 51, wherein the polymerase domain is a reverse transcriptase.

[2521] Embodiment 74. The method of embodiment 73, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2522] Embodiment 75. The method of embodiment 73, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2523] Embodiment 76. The method of embodiment 73, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[2524] Embodiment 77. The method of embodiment 73, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

Embodiment 78. The polypeptide of embodiment 8, wherein the split site is between 1023 and 1024 of SEQ ID NO: 18, or at a corresponding position in an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 99%, or at least 99.5% sequence identity with SEQ ID NO: 18.

GROUP G. PE METHODS FOR MODIFYING RNA STRUCTURE/FUNCTION

[2525] Embodiment 1. A method of installing a ribonucleotide motif or tag in an RNA of interest encoded by a target nucleotide sequence by prime editing, the method comprising:

(a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase, and (ii) a PEgRNA comprising an edit template encoding the ribonucleotide motif or tag; thereby polymerizing a single strand DNA sequence encoding the ribonucleotide motif or tag; and incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a target nucleotide sequence that encodes a modified RNA of interest comprising the ribonucleotide motif or tag.

[2526] Embodiment 2. The method of embodiment 1, wherein ribonucleotide motif or tag is a detection moiety.

[2527] Embodiment 3. The method of embodiment 1, wherein the ribonucleotide motif or tag affects the expression level of the RNA of interest.

[2528] Embodiment 4. The method of embodiment 1, wherein the ribonucleotide motif or tag affects the transport or subcellular location of the RNA of interest.

[2529] Embodiment 5. The method of embodiment 1, wherein the ribonucleotide motif or tag is selected from the group consisting of SV40 type 1, SV40 type 2, SV40 type 3, hGH, BGH, rbGlob, TK, MALAT1 ENE-mascRNA, KSHV PAN ENE, Smbox/U1 snRNA box, U1 snRNA 3' box, tRNA-lysine, broccoli aptamer, spinach aptamer, mango aptamer, HDV ribozyme, and m6A.

[2530] Embodiment 6. The method of embodiment 1, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777 (see Table).

[2531] Embodiment 7. The method of embodiment 1, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

[2532] Embodiment 8. The method of embodiment 1, wherein the napDNAbp is a Cas9 nickase (nCas9).

[2533] Embodiment 9. The method of embodiment 1, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

GROUP H. PE METHODS FOR MAKING GENE LIBRARIES

[2534] Embodiment 1. A method of constructing a programmed mutant gene library by prime editing, the method comprising:

[2535] (a) contacting a library of target nucleotide sequences each comprising one or more target genetic loci with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase, and (ii) a PEgRNA comprising an edit template comprising a sequence having at least one genetic change relative to the one or more target genetic loci;

[2536] thereby polymerizing a single strand DNA sequence templated by the edit template; and

[2537] incorporating the single strand DNA sequence in place of the one or more target genetic loci through a DNA repair and/or replication process, thereby incorporating the at least one genetic change into the target genetic loci of the target nucleotide sequences of said library.

[2538] Embodiment 2. The method of embodiment 1, wherein the library is a plasmid library.

[2539] Embodiment 3. The method of embodiment 1, wherein the library is a phage library.

[2540] Embodiment 4. The method of embodiment 1, wherein the one or more target genetic loci comprise a region encoding a protein.

[2541] Embodiment 5. The method of embodiment 1, wherein the one or more target genetic loci comprise a region encoding a secondary structure motif of a protein.

[2542] Embodiment 6. The method of embodiment 5, wherein the secondary structure motif is an alpha helix.

[2543] Embodiment 7. The method of embodiment 5, wherein the secondary structure motif is a beta sheet.

[2544] Embodiment 8. The method of embodiment 1, wherein the napDNAbp is a Cas9 protein or variant thereof.

[2545] Embodiment 9. The method of embodiment 1, wherein the napDNAbp is a nuclease with a nickase activity.

[2546] Embodiment 10. The method of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2547] Embodiment 11. The method of embodiment 1, wherein the napDNAbp comprises an amino acid sequence of SEQ ID NO: 18.

[2548] Embodiment 12. The method of embodiment 1, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2549] Embodiment 13. The method of embodiment 1, wherein the polymerase domain is a reverse transcriptase.

[2550] Embodiment 14. The method of embodiment 13, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2551] Embodiment 15. The method of embodiment 14, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2552] Embodiment 16. The method of embodiment 1, wherein the at least one genetic change in the edit template is an insertion.

[2553] Embodiment 17. The method of embodiment 1, wherein the at least one genetic change in the edit template is a deletion.

[2554] Embodiment 18. The method of embodiment 1, wherein the at least one genetic change in the edit template is substitution.

[2555] Embodiment 19. The method of embodiment 1, wherein the at least one genetic change is an insertion of one or more codons.

[2556] Embodiment 20. The method of embodiment 1, wherein the at least one genetic change is a deletion of one or more codons.

[2557] Embodiment 21. The method of embodiment 1, wherein the at least one genetic change is the insertion of a stop codon.

[2558] Embodiment 22. The method of embodiment 1, wherein the at least one genetic change is the conversion of a non-stop codon to a stop codon.

[2559] Embodiment 23. The method of embodiment 1, wherein the method is conduct simultaneously at least 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, or 10, or between 10-100, or between 100-200, or between 200-300, or between 300-400, or between 400-500 target genetic loci in each of the target nucleotide sequences of the library.

[2560] Embodiment 24. The method of embodiment 1, wherein the method is conducted during PACE or PANCE evolution, and wherein each instance of incorporating the at least one genetic change into the target genetic loci of the target nucleotide sequences of said library also installs a new target sequence.

GROUP I. PE METHODS FOR OFF-TARGET DETECTION

[2561] Embodiment 1. A method of evaluating off-target editing by a prime editor, the method comprising:

[2562] (a) contacting a target nucleotide sequence having an edit site with a (i) prime editor fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising a DNA synthesis template that encodes a detectable sequence;

[2563] wherein the PEgRNA complexes with the fusion protein and guides said fusion protein to the edit site and, if present, to one or more off-target sites;

[2564] and wherein the prime editor fusion protein installs the detectable sequence at the edit site, and, if present, at the one or more off-target sites;

[2565] (b) determining the nucleotide sequence of the edit site and the one or more off-target sites.

[2566] Embodiment 2. The method of embodiment 1, wherein the target nucleotide sequence is a genome.

[2567] Embodiment 3. The method of embodiment 1, wherein the step of contacting is in vitro.

[2568] Embodiment 4. The method of embodiment 1, wherein the step of contacting is in vivo.

[2569] Embodiment 5. The method of embodiment 1, wherein the edit site is a mutation in a disease-associated gene.

[2570] Embodiment 6. The method of embodiment 5, wherein the mutation is a single base substitution, insertion, deletion, or inversion.

[2571] Embodiment 7. The method of embodiment 1, wherein the disease-associated gene is associated with a monogentic disorder selected from the group consisting of: Adenosine Deaminase (ADA) Deficiency; Alpha-1 Antitrypsin Deficiency; Cystic Fibrosis; Duchenne Muscular Dystrophy; Galactosemia; Hemochromatosis; Huntington's Disease;

Maple Syrup Urine Disease; Marfan Syndrome; Neurofibromatosis Type 1; Pachyonychia Congenita; Phenylkeotnuria; Severe Combined Immunodeficiency; Sickle Cell Disease; Smith-Lemli-Opitz Syndrome; and Tay-Sachs Disease.

[2572] Embodiment 8. The method of embodiment 1, wherein the disease-associated gene is associated with a polygenic disorder selected from the group consisting of: heart disease; high blood pressure; Alzheimer's disease; arthritis; diabetes; cancer; and obesity.

[2573] Embodiment 9. The method of embodiment 1, wherein the fusion protein has an amino acid sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

[2574] Embodiment 10. The method of embodiment 1, wherein the napDNAbp is Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute, or a variant of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute.

[2575] Embodiment 11. The method of embodiment 1, wherein the napDNAbp is a Cas9 or variant thereof.

[2576] Embodiment 12. The method of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2577] Embodiment 13. The method of embodiment 1, wherein the napDNAbp is Cas9 nickase (nCas9).

[2578] Embodiment 14. The method of embodiment 1, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[2579] Embodiment 15. The method of embodiment 1, wherein the napDNAbp is SpCas9 wild type or a variant thereof of any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2580] Embodiment 16. The method of embodiment 1, wherein the napDNAbp is an SpCas9 ortholog.

[2581] Embodiment 17. The method of embodiment 1, wherein the napDNAbp is any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2582] Embodiment 18. The method of embodiment 1, wherein the napDNAbp comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2583] Embodiment 19. The method of embodiment 1, wherein the polymerase comprises an RNA-dependent DNA polymerase activity.

[2584] Embodiment 20. The method of embodiment 1, wherein the polymerase is a reverse transcriptase.

[2585] Embodiment 21. The method of embodiment 1, wherein the reverse transcriptase is a naturally occuring wild type reverse transcriptase having an amino acid sequence of any one of SEQ ID NOs: 89 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89.

[2586] Embodiment 22. The method of embodiment 1, wherein the reverse transcriptase is a variant reverse transcriptase having an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2587] Embodiment 23. The method of embodiment 1, wherein the reverse transcriptase comprises any one of the amino acid sequences of SEQ ID NO: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2588] Embodiment 24. The method of embodiment 1, wherein the reverse transcriptase comprises an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2589] Embodiment 25. The method of embodiment 1, wherein the detectable sequence is an insertion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or

at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2590] Embodiment 26. The method of embodiment 1, wherein the detectable sequence is a deletion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2591] Embodiment 27. The method of embodiment 1, wherein the detectable sequence is a nucleobase substitution.

[2592] Embodiment 28. The method of embodiment 1, wherein the detectable sequence is a transition mutation.

[2593] Embodiment 29. The method of embodiment 1, wherein the detectable sequence is a transversion mutation.

[2594] Embodiment 30. The method of embodiment 1, wherein the detectable sequence is a single nucleotide substitution selected from the group consisting of: (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, and (12) an A to C substitution.

[2595] Embodiment 31. The method of embodiment 1, wherein the detectable sequence is a single nucleotide substitution that converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2596] Embodiment 32. The method of embodiment 1, wherein the detectable sequence is a barcode sequence.

[2597] Embodiment 33. The method of embodiment 1, wherein step (d) of determining the nucleotide sequence at the on-target and off-target sites comprises (i) fragmenting the target nucleotide sequence to form fragments, (ii) attaching adapter sequences to the ends of the fragments, (iii) PCR amplifying an amplicon using a pair of primers wherein one primer anneals to an adapter sequence attached at one end of the fragments, and another primer that anneals to an adapter sequence inserted by prime editing located within the fragment, and (iv) sequencing the amplicon to determine the location of the edit.

GROUP J. PE METHODS FOR CELL DATA RECORDING

[2598] Embodiment 1. A method of recording a cellular event by prime editing, the method comprising: (A) introducing into a cell one or more constructs encoding (i) a prime editor fusion protein comprising an napDNAbp and an RNA-dependent DNA polymerase and (ii) a PEgRNA, wherein the expression of the fusion protein and/or the PEgRNA is induced by the occurrence of a cellular event, and wherein upon expression of the fusion protein and/or the PEgRNA results in prime editing of a target edit site in the genome of the cell to introduce a detectable sequence, and (B) identifying the detectable sequence, thereby identifying the occurrence of the cellular event.

[2599] Embodiment 2. The method of embodiment 1, wherein the prime editing of step (A) simultaneously introduces a new target edit site such that the recording of the cellular event may occur iteratively.

[2600] Embodiment 3. The method of embodiment 1, wherein the PEgRNA comprises an edit template that encodes the detectable sequence.

[2601] Embodiment 4. The method of embodiment 3, wherein the edit template further encodes a new target edit site.

[2602] Embodiment 5. The method of embodiment 1, wherein the detectable sequence is an insertion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at

least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2603] Embodiment 6. The method of embodiment 1, wherein the detectable sequence is a deletion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2604] Embodiment 7. The method of embodiment 1, wherein the detectable sequence is a nucleobase substitution.

[2605] Embodiment 8. The method of embodiment 1, wherein the detectable sequence is a transition mutation.

[2606] Embodiment 9. The method of embodiment 1, wherein the detectable sequence is a transversion mutation.

[2607] Embodiment 10. The method of embodiment 1, wherein the detectable sequence is a single nucleotide substitution of wherein the single nucleotide substitution is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[2608] Embodiment 11. The method of embodiment 1, wherein the detectable sequence is a single nucleotide substitution that converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2609] Embodiment 12. The method of embodiment 1, wherein the detectable sequence is a barcode sequence.

[2610] Embodiment 13. The method of embodiment 1, wherein the detectable sequence increases in length over time as a result of iterative insertion of the detectable sequence for each occurrence of the cellular event.

[2611] Embodiment 14. The method of embodiment 1, wherein the detecting step comprises sequencing the edited target site, or an amplicon of the edited target site.

[2612] Embodiment 15. The method of embodiment 1, wherein the napDNAbp is Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute, or a variant of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute.

[2613] Embodiment 16. The method of embodiment 1, wherein the napDNAbp is a Cas9 or variant thereof.

[2614] Embodiment 17. The method of embodiment 1, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2615] Embodiment 18. The method of embodiment 1, wherein the napDNAbp is Cas9 nickase (nCas9).

[2616] Embodiment 19. The method of embodiment 1, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[2617] Embodiment 20. The method of embodiment 1, wherein the napDNAbp is SpCas9 wild type or a variant thereof of any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2618] Embodiment 21. The method of embodiment 1, wherein the napDNAbp is an SpCas9 ortholog.

[2619] Embodiment 22. The method of embodiment 1, wherein the napDNAbp is any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2620] Embodiment 23. The method of embodiment 1, wherein the RNA-dependent DNA polymerase is a reverse transcriptase.

[2621] Embodiment 24. The method of embodiment 23, wherein the reverse transcriptase is a naturally occuring wild type reverse transcriptase having an amino acid

sequence of any one of SEQ ID NOs: 89 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89.

[2622] Embodiment 25. The method of embodiment 23, wherein the reverse transcriptase is a variant reverse transcriptase having an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2623] Embodiment 26. The method of embodiment 1, wherein the fusion protein comprises an amino acid sequence of any one of SEQ ID NOs: 123 and 134 (PE1, PE2), or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs:123 and 134 (PE1, PE2).

[2624] Embodiment 27. The method of embodiment 1, wherein the one or more constructs that encode the prime editor fusion protein and/or the PEgRNA further comprises one or more promoters which are inducible when cellular event occurs.

[2625] Embodiment 28. The method of embodiment 1 , wherein the cellular event is marked by a stimulus received by the cell.

[2626] Embodiment 29. The method of embodiment 28, wherein the stimulus is a small molecule, a protein, a peptide, an amino acid, a metabolite, an inorganic molecule, an organometallic molecule, an organic molecule, a drug or drug candidate, a sugar, a lipid, a metal, a nucleic acid, a molecule produced during the activation of an endogenous or an exogenous signaling cascade, light, heat, sound, pressure, mechanical stress, shear stress, or a virus or other microorganism, change in pH, or change in oxidation/reduction state.

[2627] Embodiment 30. A cell data recording plasmid for recording a cellular event using prime editing, comprising:

[2628] i. a fusion protein comprising (a) a nucleic acid sequence encoding a nucleic acid programmable DNA binding protein (napDNAbp) and a (b) RNA-dependent DNA polymerase, said fusion protein being operably linked to a first promoter;

[2629] ii. a nucleic acid sequence encoding a prime editor guide RNA (PEgRNA) operably linked to a second promoter, wherein the PEgRNA is complementary to a target sequence; and

[2630] iii. an origin of replication;

[2631] wherein at least one of the promoters is an inducible promoter, and wherein the PEgRNA associates with the napDNAbp under conditions that induce expression of the PEgRNA and expression of the napDNAbp sufficiently to install a detectable sequence at a target edit site.

[2632] Embodiment 31. The cell data recording plasmid of embodiment 30, wherein the inducible promoter is induced by the cellular event.

[2633] Embodiment 32. The cell data recording plasmid of embodiment 30, wherein the cellular event is marked by a stimulus received by the cell.

[2634] Embodiment 33. The cell data recording plasmid of embodiment 32, wherein the stimulus is a small molecule, a protein, a peptide, an amino acid, a metabolite, an inorganic molecule, an organometallic molecule, an organic molecule, a drug or drug candidate, a sugar, a lipid, a metal, a nucleic acid, a molecule produced during the activation of an endogenous or an exogenous signaling cascade, light, heat, sound, pressure, mechanical stress, shear stress, or a virus or other microorganism, change in pH, or change in oxidation/reduction state.

[2635] Embodiment 34. The cell data recording plasmid of embodiment 30, wherein the first and second promoter are the same.

[2636] Embodiment 35. The cell data recording plasmid of embodiment 30, wherein the first and second promoter are different.

[2637] Embodiment 36. The cell data recording plasmid of embodiment 30, wherein the at least one inducible promoter is an anhydrotetracycline-inducible promoter, IPTG-inducible promoter, rhamnose-inducible promoter, or arabinose-inducible promoter.

[2638] Embodiment 37. The cell data recording plasmid of embodiment 30, wherein the first or second promoter is a constitutive promoter.

[2639] Embodiment 38. The cell data recording plasmid of embodiment 37, wherein the constitutive promoter is a Lac promoter, cytomegalovirus (CMV) promoter, a constitutive RNA polymerase III promoter, or a UBC promoter.

[2640] Embodiment 39. The cell data recording plasmid of embodiment 30, wherein the origin of replication comprises a pSC101, pMB1, pBR322, ColE1, or p15A origin of replication sequence.

[2641] Embodiment 40. The cell data recording plasmid of embodiment 30, wherein the PEgRNA comprises an edit template that encodes the detectable sequence.

[2642] Embodiment 41. The cell data recording plasmid of embodiment 40, wherein the edit template further encodes a new target edit site.

[2643] Embodiment 42. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is an insertion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2644] Embodiment 43. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a deletion of at least 1, or at least 2, or at least 3, or at least 4, or at least 5, or at least 6, or at least 7, or at least 8, or at least 9, or at least 10, or at least 11, or at least 12, or at least 13, or at least 14, or at least 15, or at least 16, or at least 17, or at least 18, or at least 19, or at least 20, or at least 21, or at least 22, or at least 23, or at least 24, or at least 25, or at least 26, or at least 27, or at least 28, or at least 29, or at least 30, or at least 31, or at least 32, or at least 33, or at least 34, or at least 35, or at least 40, or at least 50, or at least 60, or at least 70, or at least 80, or at least 90, or at least 100 nucleobases.

[2645] Embodiment 44. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a nucleobase substitution.

[2646] Embodiment 45. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a transition mutation.

[2647] Embodiment 46. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a transversion mutation.

[2648] Embodiment 47. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a single nucleotide substitution of wherein the single nucleotide substitution is (1) a G to T substitution, (2) a G to A substitution, (3) a G to C substitution, (4) a T to G substitution, (5) a T to A substitution, (6) a T to C substitution, (7) a C to G substitution, (8) a C to T substitution, (9) a C to A substitution, (10) an A to T substitution, (11) an A to G substitution, or (12) an A to C substitution.

[2649] Embodiment 48. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a single nucleotide substitution that converts (1) a G:C basepair to a T:A basepair, (2) a G:C basepair to an A:T basepair, (3) a G:C basepair to C:G basepair, (4) a

T:A basepair to a G:C basepair, (5) a T:A basepair to an A:T basepair, (6) a T:A basepair to a C:G basepair, (7) a C:G basepair to a G:C basepair, (8) a C:G basepair to a T:A basepair, (9) a C:G basepair to an A:T basepair, (10) an A:T basepair to a T:A basepair, (11) an A:T basepair to a G:C basepair, or (12) an A:T basepair to a C:G basepair.

[2650] Embodiment 49. The cell data recording plasmid of embodiment 30, wherein the detectable sequence is a barcode sequence.

[2651] Embodiment 50. The cell data recording plasmid of embodiment 30, wherein the detectable sequence increases in length over time as a result of iterative insertion of the detectable sequence for each occurrence of the cellular event.

[2652] Embodiment 51. The cell data recording plasmid of embodiment 30, wherein the detecting step comprises sequencing the edited target site, or an amplicon of the edited target site.

[2653] Embodiment 52. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute, or a variant of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute.

[2654] Embodiment 53. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is a Cas9 or variant thereof.

[2655] Embodiment 54. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

[2656] Embodiment 55. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is Cas9 nickase (nCas9).

[2657] Embodiment 56. The cell data recording plasmid of embodiment 30, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with SEQ ID NO: 18.

[2658] Embodiment 57. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is SpCas9 wild type or a variant thereof of any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2659] Embodiment 58. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is an SpCas9 ortholog.

[2660] Embodiment 59. The cell data recording plasmid of embodiment 30, wherein the napDNAbp is any one of amino acid sequences 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

[2661] Embodiment 60. The cell data recording plasmid of embodiment 30, wherein the RNA-dependent DNA polymerase is a reverse transcriptase.

[2662] Embodiment 61. The cell data recording plasmid of embodiment 30, wherein the reverse transcriptase is a naturally occuring wild type reverse transcriptase having an amino acid sequence of any one of SEQ ID NOs: 89 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89.

[2663] Embodiment 62. The cell data recording plasmid of embodiment 30, wherein the reverse transcriptase is a variant reverse transcriptase having an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, 235, 454, 471, 516, 662, 700, 701-716, 739-741, and 766.

[2664] Embodiment 63. The cell data recording plasmid of embodiment 30, wherein the fusion protein comprises an amino acid sequence of any one of SEQ ID NOs: 123 and 134 (PE1, PE2), or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with any of SEQ ID NOs: 123 and 134 (PE1, PE2).

[2665] Embodiment 64. A kit for use in a cell comprising the cell data recorder plasmid of any one of embodiments 30 63.

[2666] Embodiment 65. The kit of embodiment 64, wherein the cell is a prokaryotic cell.

[2667] Embodiment 66. The kit of embodiment 64, wherein the cell is a eukaryotic cell.

[2668] Embodiment 67. A cell comprising the cell data recording plasmid of any one of embodiments 30-63.

[2669] Embodiment 68. The cell of embodiment 67, wherein the cell is a prokaryotic cell.

[2670] Embodiment 69. The cell of embodiment 67, wherein the cell is a eukaryotic cell.

[2671] Embodiment 70. The cell of embodiment 69, wherein the eukaryotic cell is a mammalian cell.

[2672] Embodiment 71. The cell of embodiment 70, wherein the mammalian cell is a human cell.

[2673] Embodiment 72. A method of recording a cellular event by prime editing, the method comprising: (A) introducing into a cell a cell data recording plasmid of any of embodiments 30-63, wherein the fusion protein and/or the PEgRNA are induced by the occurrence of a cellular event, and wherein expression of the fusion protein and/or the PEgRNA results in prime editing of a target edit site in the genome of the cell to introduce a detectable sequence, and (B) identifying the detectable sequence, thereby identifying the occurrence of the cellular event.

[2674] Embodiment 73. The method of embodiment 72, wherein the step of (A) introducing is by transfection or electroporation.

EQUIVALENTS AND SCOPE

[2675] In the articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Embodiments or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

[2676] Furthermore, the disclosure encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claims that is dependent on the same base claim. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should it be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements and/or features, certain embodiments of the disclosure or aspects of the disclosure consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that the terms "comprising" and "containing" are intended to be open and permits the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub–range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[2677] This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the embodiments. Because such embodiments are deemed to be known to one of ordinary skill in

the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the invention can be excluded from any embodiment, for any reason, whether or not related to the existence of prior art.

[2678] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The scope of the present embodiments described herein is not intended to be limited to the above Description, but rather is as set forth in the appended embodiments. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following embodiments.

CLAIMS

What is claimed is:

1. A method for mutagenizing a DNA molecule at a target locus by prime editing, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone polymer (e.g., error-prone reverse transcriptase), and (ii) a guide RNA comprising an edit template comprising a desired nucleotide change; thereby polymerizing a single stranded DNA templated from the edit template; and incorporating the single stranded DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

2. The method of any prior claim, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

3. The method of any prior claim, wherein the napDNAbp is a Cas9 nickase (nCas9).

4. The method of claim 1, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

5. The method of claim 1, wherein the guide RNA comprises SEQ ID NOs: 222.

6. A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety;

thereby polymerizing a single strand DNA sequence encoding the immunoepitope;

thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

7. The method of claim 6, wherein the immunoepitope is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoeptitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); and neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

8. The method of claim 6, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

9. The method of claim 6, wherein the napDNAbp is a Cas9 nickase (nCas9).

10. The method of claim 6, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

11. The method of claim 6, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

12. A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase, and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain;

thereby polymerizing a single strand DNA sequence encoding the immunoepitope;

thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;

wherein the method produces a modified target nucleotide sequence that encodes a

fusion protein comprising the protein of interest and the small molecule dimerization domain.

13. The method of claim 12 further comprising conducting the method on a second protein of interest.

14. The method of claim 13, wherein the first protein of interest and the second protein of interest dimerize in the presence of a small molecule that binds to the dimerization domain on each of said proteins.

15. The method of claim 12, wherein the small molecule binding domain is FKBP12 of SEQ ID NO: 488.

16. The method of claim 12, wherein the small molecule binding domain is FKBP12-F36V of SEQ ID NO: 489.

17. The method of claim 12, wherein the small molecule binding domain is cyclophilin of SEQ ID NOs: 490 and 493-494.

18. The method of claim 12, wherein the small molecule is a dimer of a small molecule as described herein.

19. The method of claim 12, wherein the fusion protein comprises the amino acid sequence of PE1, PE2, or PE3.

20. The method of claim 12, wherein the napDNAbp is a Cas9 nickase (nCas9).

21. The method of claim 12, wherein the napDNAbp comprises the amino acid sequence of SEQ ID NOs: 18-88, 126, 130, 137, 141, 147, 153, 157, 445, 460, 467, and 482-487.

22. The method of claim 12, wherein the PEgRNA comprises SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

23. A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are

expressed from the recombinant nucleotide sequence as a fusion protein.

24. The method of claim 23, wherein the peptide tag is used for purification and/or detection of the protein.

25. The method of claim 23, wherein the peptide tag is a poly-histidine (e.g., HHHHHH) (SEQ ID NO: 252-262), FLAG (e.g., DYKDDDDK) (SEQ ID NO: 2), V5 (e.g., GKPIPNPLLGLDST) (SEQ ID NO: 3), GCN4, HA (e.g., YPYDVPDYA) (SEQ ID NO: 5), Myc (e.g. EQKLISEED) (SEQ ID NO: 6), or GST.

26. The method of claim 23, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 1-6, 245-249, 252-262, 264-273, 275-276, 281, 278-288, and 622.

27. The method of claim 23, wherein the peptide tag is fused to the protein by a linker.

28. The method of claim 23, wherein the fusion protein has the following structure: [protein]-[peptide tag] or [peptide tag]-[protein], wherein "]-[" represents an optional linker.

29. The method of claim 23, wherein the linker has an amino acid sequence of SEQ ID NO: 127, 165-176, 446, 453, and 767-769.

30. The method of claim 23, wherein the prime editor construct comprises a PEgRNA comprising the nucleotide sequence of SEQ ID NOs: 101-104, 181-183, 223-244, 277, 325-334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 499-505, 735-761, 776-777.

31. The method of claim 23, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

32. The method of claim 23, wherein the PEgRNA comprises a spacer, a gRNA core, and an extension arm, wherein the spacer is complementary to the target nucleotide sequence and the extension arm comprises a reverse transcriptase template that encodes the peptide tag.

33. A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the

target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

34. The method of claim 33, wherein functional moiety alters the phosphorylation, ubiquitylation, glycosylation, lipidation, hydroxylation, methylation, acetylation, crotonylation, or SUMOylation state of the protein of interest.

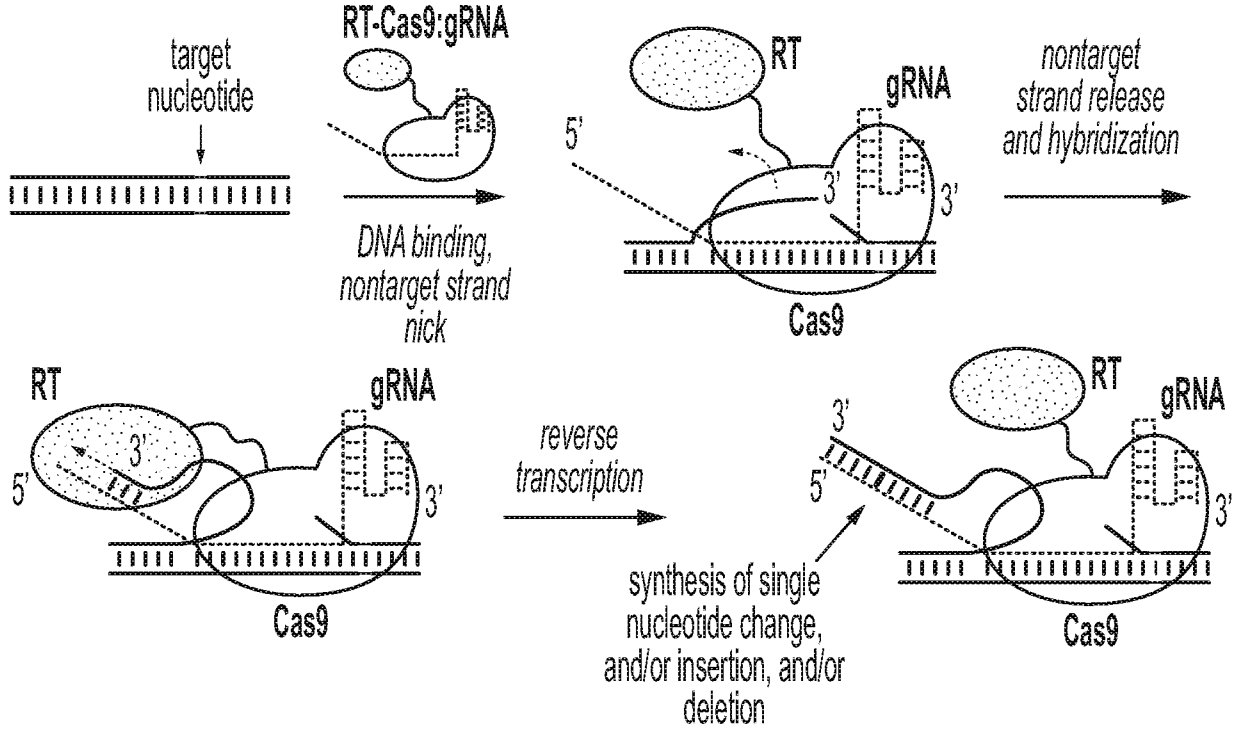

target nucleotide
RT-Cas9:gRNA
DNA binding, nontarget strand nick
RT
gRNA
5'
3'
3'
Cas9
nontarget strand release and hybridization
RT
gRNA
5'
3'
3'
Cas9
reverse transcription
3'
5'
RT
gRNA
3'
Cas9
synthesis of single nucleotide change, and/or insertion, and/or deletion

FIG. 1A

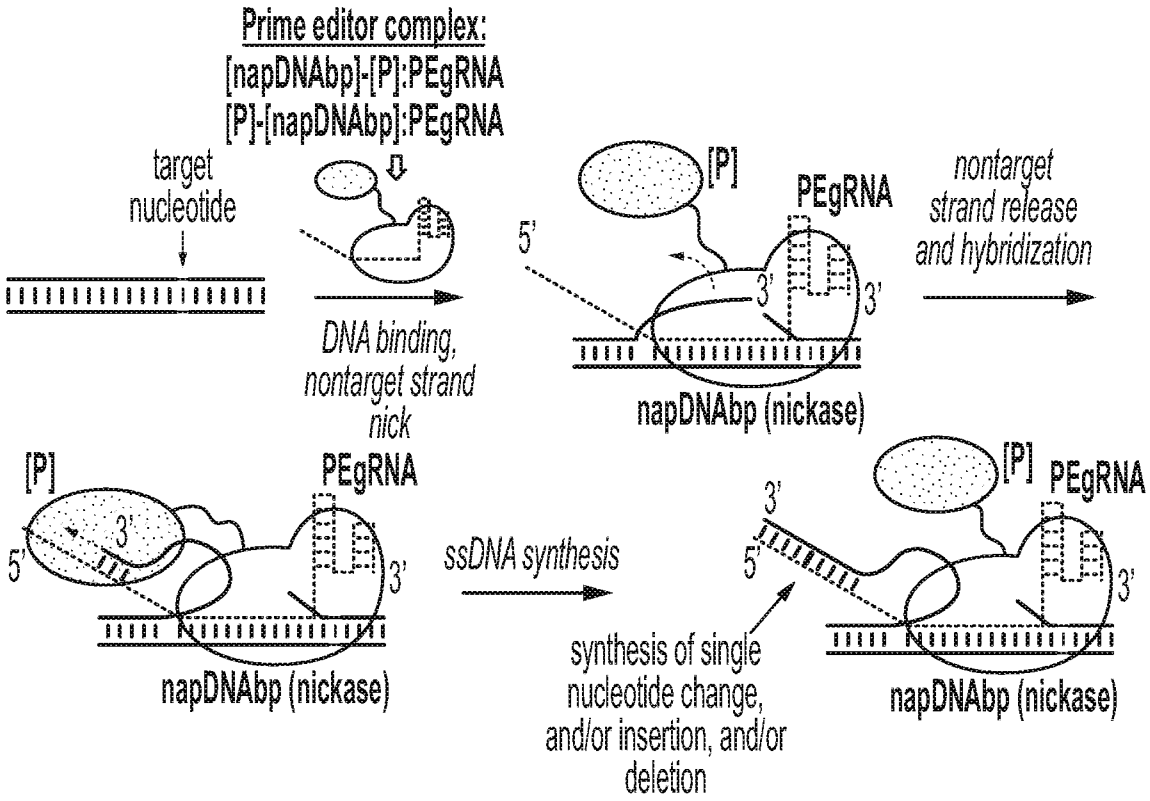

Prime editor complex:
[napDNAbp]-[P]:PEgRNA
[P]-[napDNAbp]:PEgRNA
target nucleotide
DNA binding, nontarget strand nick
[P]
PEgRNA
5'
3'
3'
napDNAbp (nickase)
nontarget strand release and hybridization
[P]
PEgRNA
5'
3'
3'
napDNAbp (nickase)
ssDNA synthesis
3'
5'
[P]
PEgRNA
3'
napDNAbp (nickase)
synthesis of single nucleotide change, and/or insertion, and/or deletion

FIG. 1B

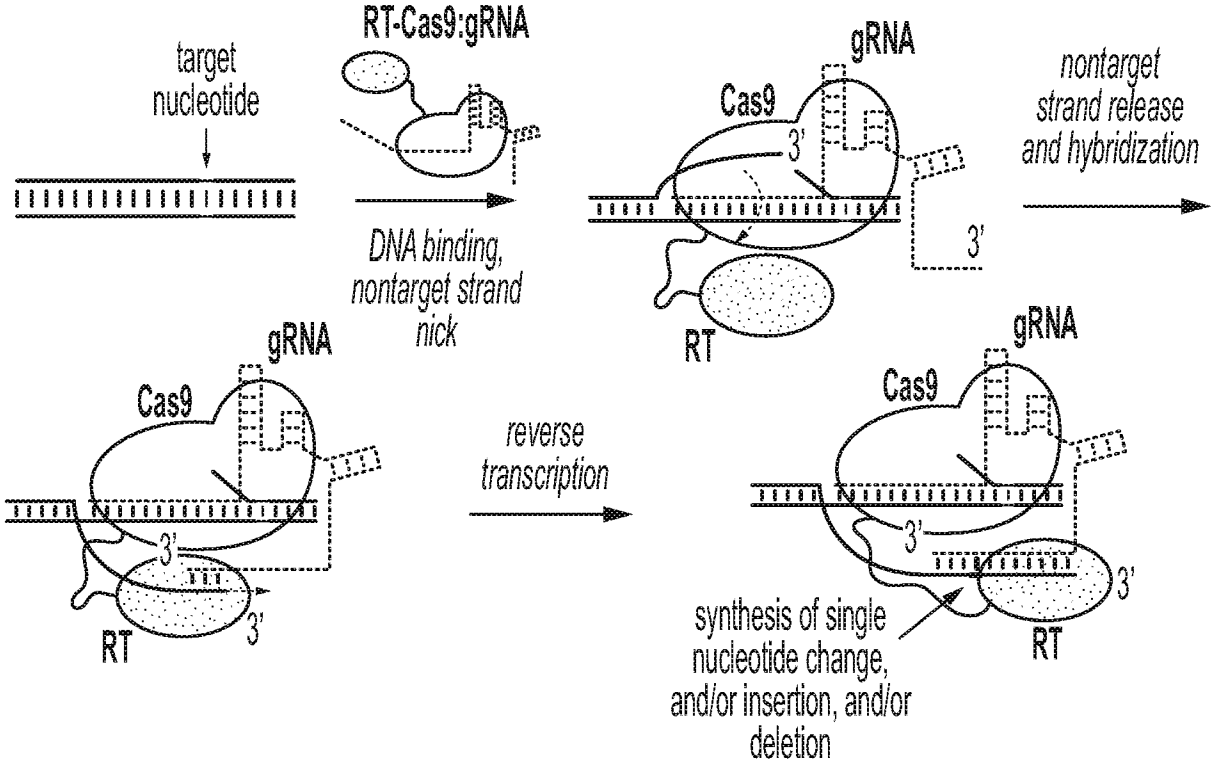

target nucleotide
RT-Cas9:gRNA
DNA binding, nontarget strand nick
gRNA
Cas9
3'
RT
nontarget strand release and hybridization
reverse transcription
synthesis of single nucleotide change, and/or insertion, and/or deletion

FIG. 1C

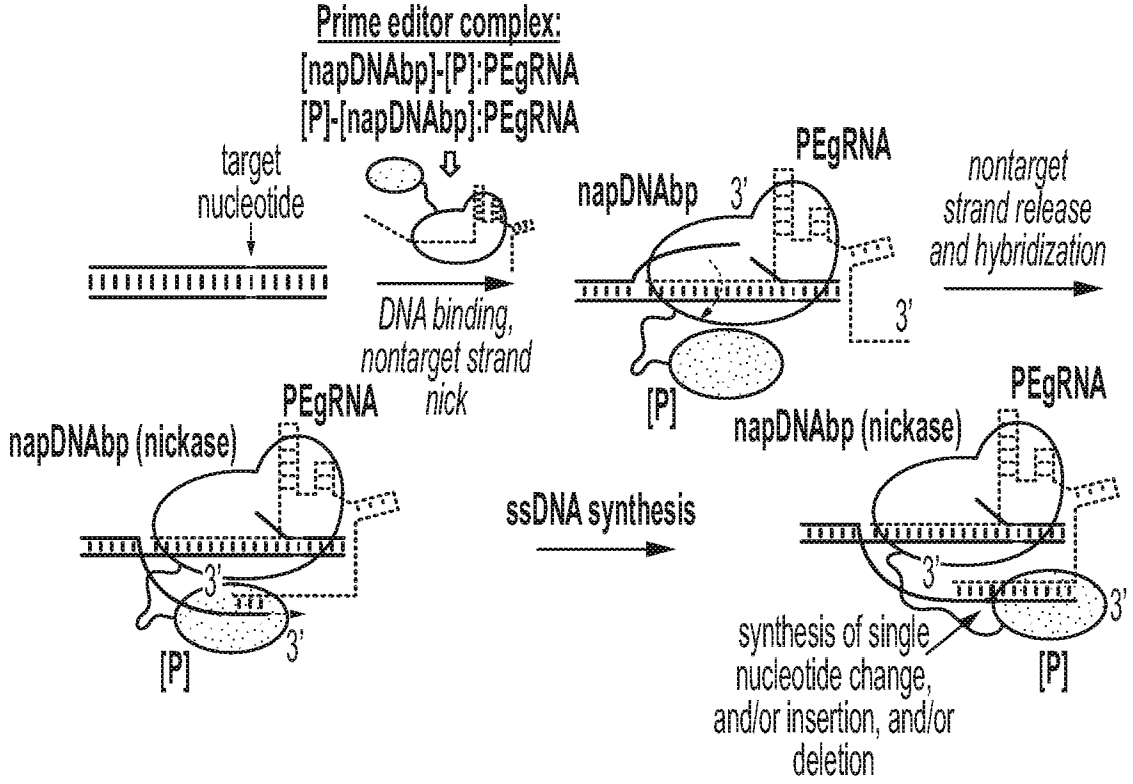

Prime editor complex:
[napDNAbp]-[P]:PEgRNA
[P]-[napDNAbp]:PEgRNA
target nucleotide
DNA binding, nontarget strand nick
PEgRNA
napDNAbp
3'
[P]
nontarget strand release and hybridization
napDNAbp (nickase)
ssDNA synthesis
synthesis of single nucleotide change, and/or insertion, and/or deletion

FIG. 1D

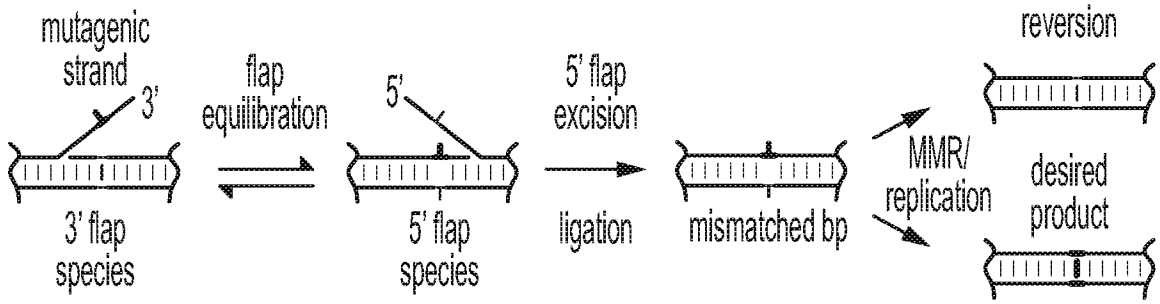

mutagenic strand
3'
3' flap species
flap equilibration
5'
5' flap species
5' flap excision
ligation
mismatched bp
MMR/ replication
reversion
desired product

FIG. 1E

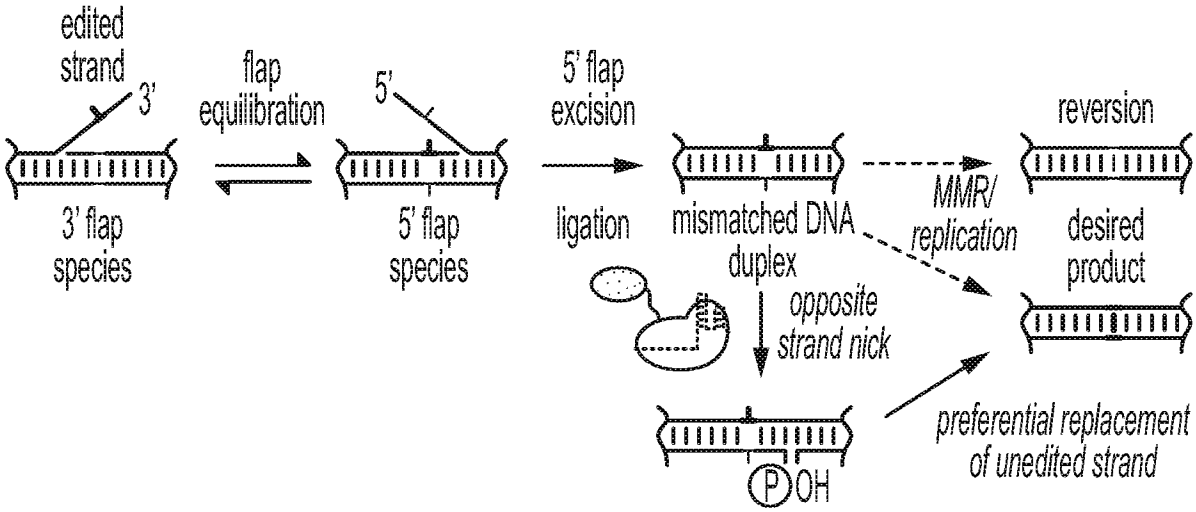

edited strand
3'
3' flap species
flap equilibration
5'
5' flap species
5' flap excision
ligation
mismatched DNA duplex
opposite strand nick
P OH
MMR/ replication
reversion
desired product
preferential replacement of unedited strand

FIG. 1F

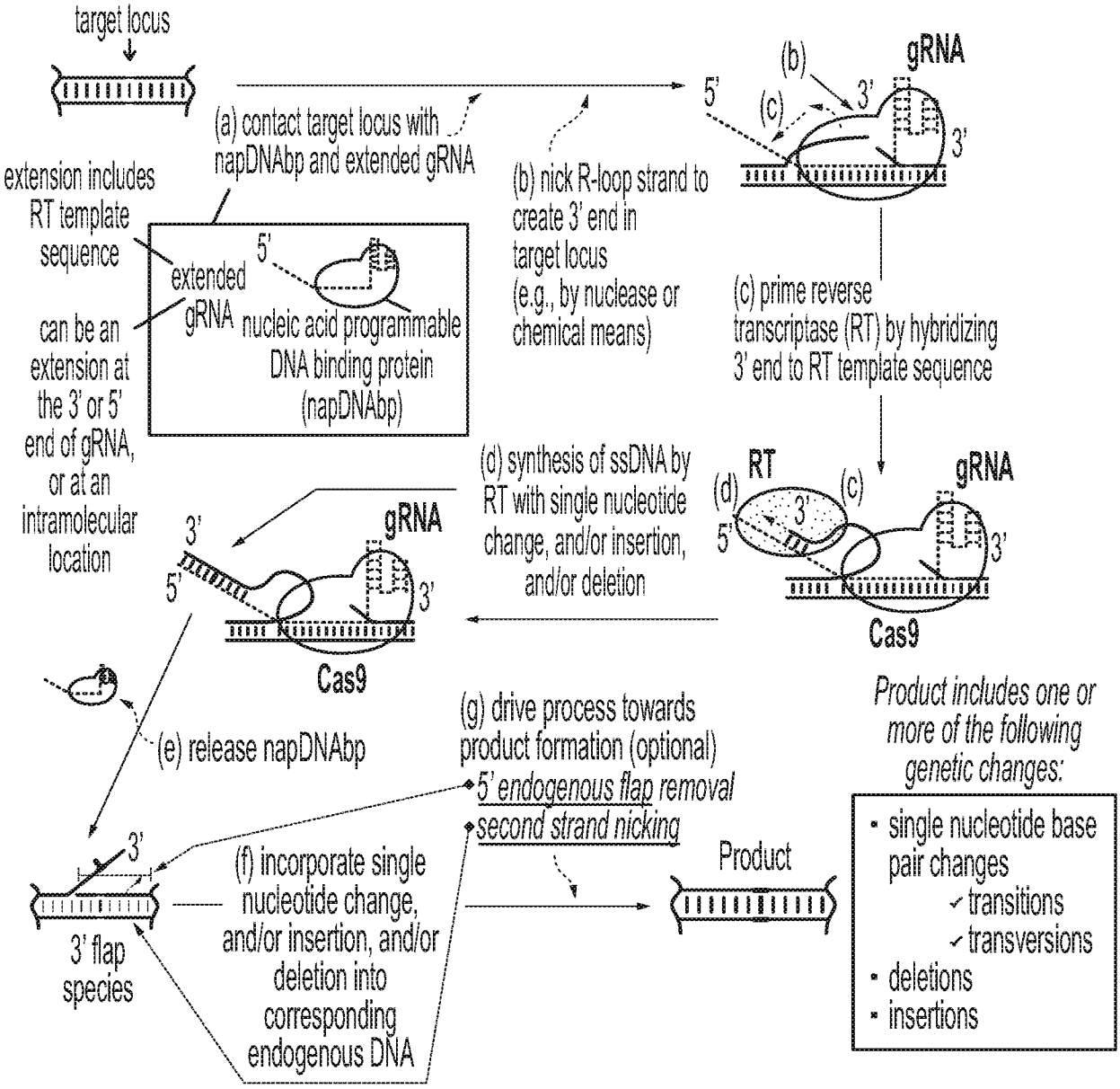

target locus
(a) contact target locus with napDNAbp and extended gRNA
extension includes RT template sequence
can be an extension at the 3' or 5' end of gRNA, or at an intramolecular location
extended gRNA
nucleic acid programmable DNA binding protein (napDNAbp)
(b) nick R-loop strand to create 3' end in target locus (e.g., by nuclease or chemical means)
(c) prime reverse transcriptase (RT) by hybridizing 3' end to RT template sequence
(d) synthesis of ssDNA by RT with single nucleotide change, and/or insertion, and/or deletion
(e) release napDNAbp
(f) incorporate single nucleotide change, and/or insertion, and/or deletion into corresponding endogenous DNA
3' flap species
(g) drive process towards product formation (optional)
5' endogenous flap removal
second strand nicking
Product
Product includes one or more of the following genetic changes:
single nucleotide base pair changes
transitions
transversions
deletions
insertions
gRNA
RT
Cas9

FIG. 1G

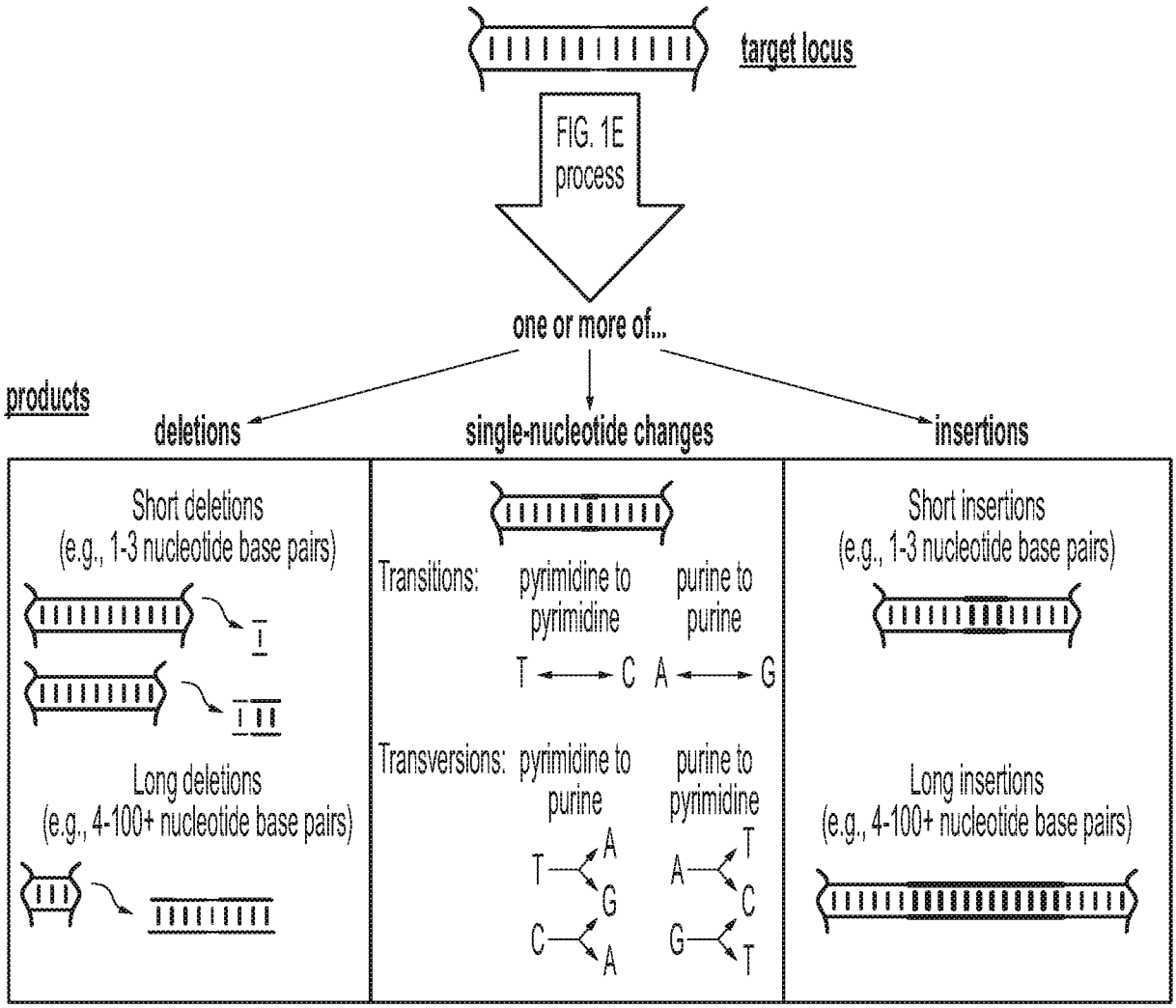

target locus
FIG. 1E process
one or more of...
products
deletions
single-nucleotide changes
insertions
Short deletions
(e.g., 1-3 nucleotide base pairs)
Long deletions
(e.g., 4-100+ nucleotide base pairs)
Transitions:
pyrimidine to pyrimidine
purine to purine
T ⟷ C A ⟷ G
Transversions:
pyrimidine to purine
purine to pyrimidine
T — A G
C — A
A — T C
G — T
Short insertions
(e.g., 1-3 nucleotide base pairs)
Long insertions
(e.g., 4-100+ nucleotide base pairs)

FIG. 1H

PE3b temporal nicking.

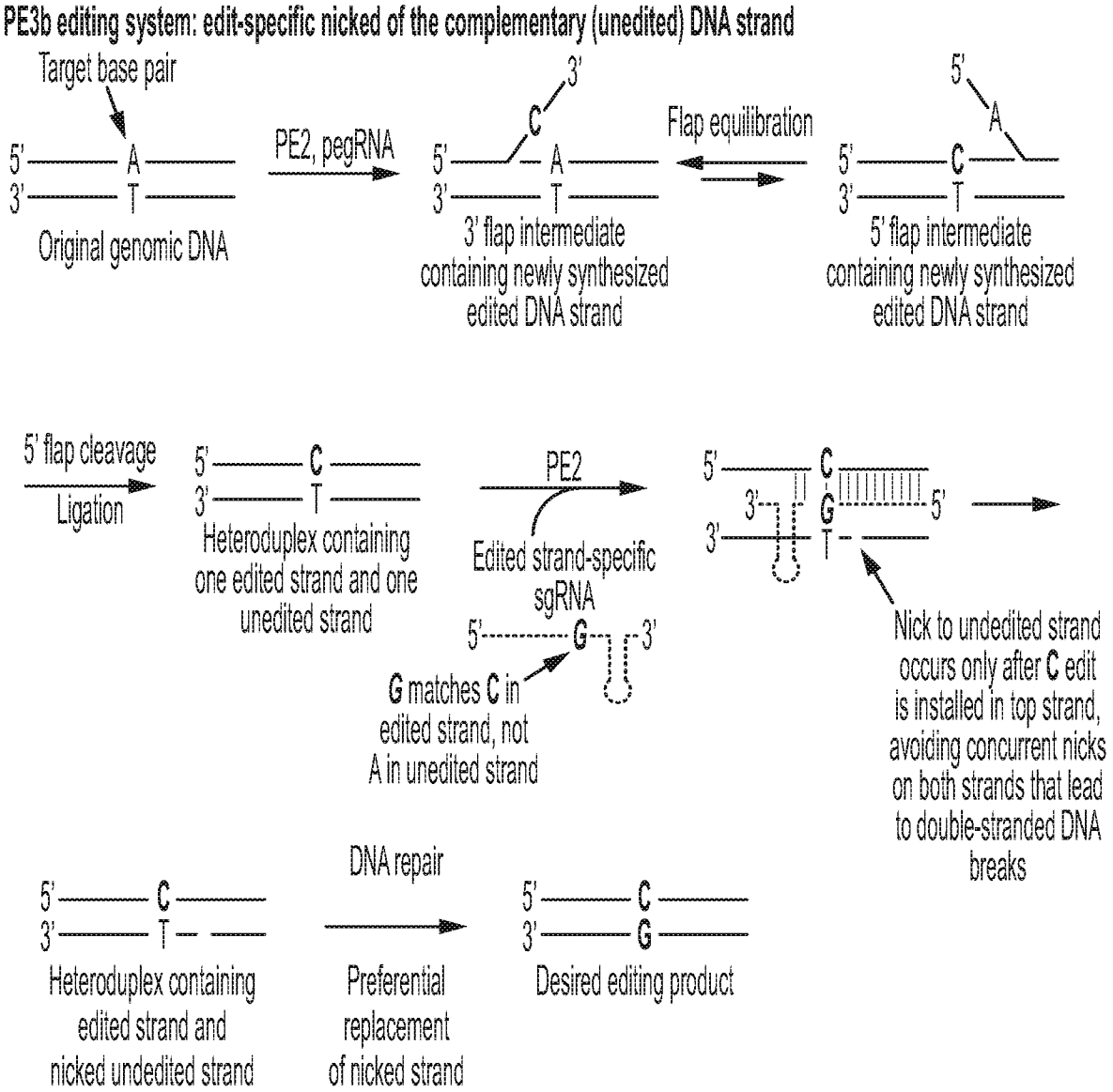


FIG. 1I

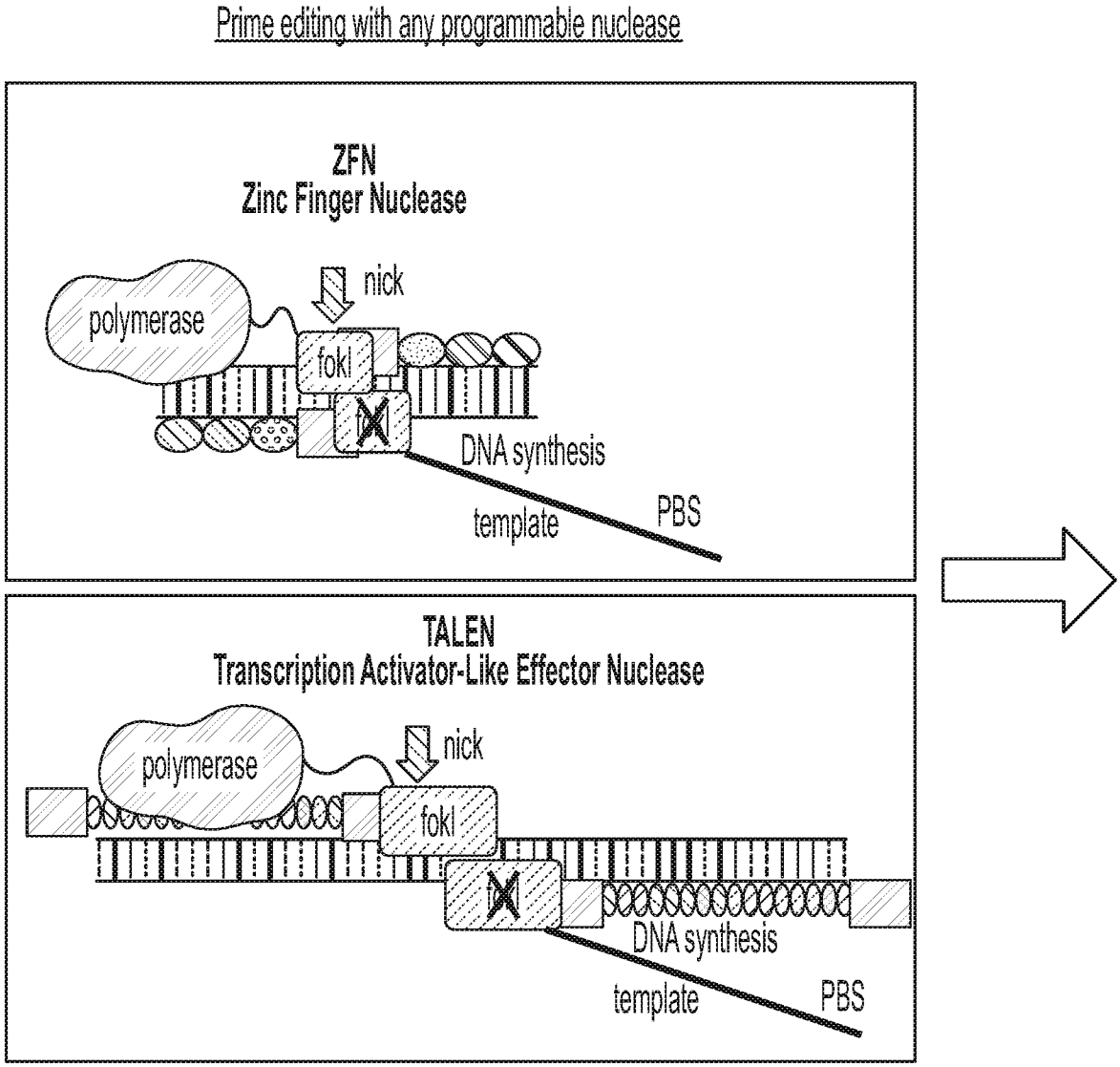

Prime editing with any programmable nuclease
ZFN
Zinc Finger Nuclease
polymerase
nick
fokI
DNA synthesis
template
PBS
TALEN
Transcription Activator-Like Effector Nuclease
polymerase
nick
fokI
DNA synthesis
template
PBS

FIG. 1J

Polymerase-dependent synthesis of a replacement strand encoded by the polymerase against the DNA synthesis template and which contains a desired edit. The mechanism is envisioned to operate similarly to prime editing. Additional factors or effectors may be added *in trans* or as fusions to facilitate the reaction as a whole (*e.g.*, (a) a helicase to unwind the DNA at the cut site to make the cut strand with the 3' end available as a primer, (b) a FEN1 to help remove the endogenous strand on the cut strand to drive the reaction towards replacement of the endogenous strand with the synthesized strand, or (c) a nCas9:gRNA complex to create a second site nick on the opposite strand, which may help drive the integration of the synthesize repair through favored cellular repair of the non-edited strand)

FIG. 1J
CONTINUED

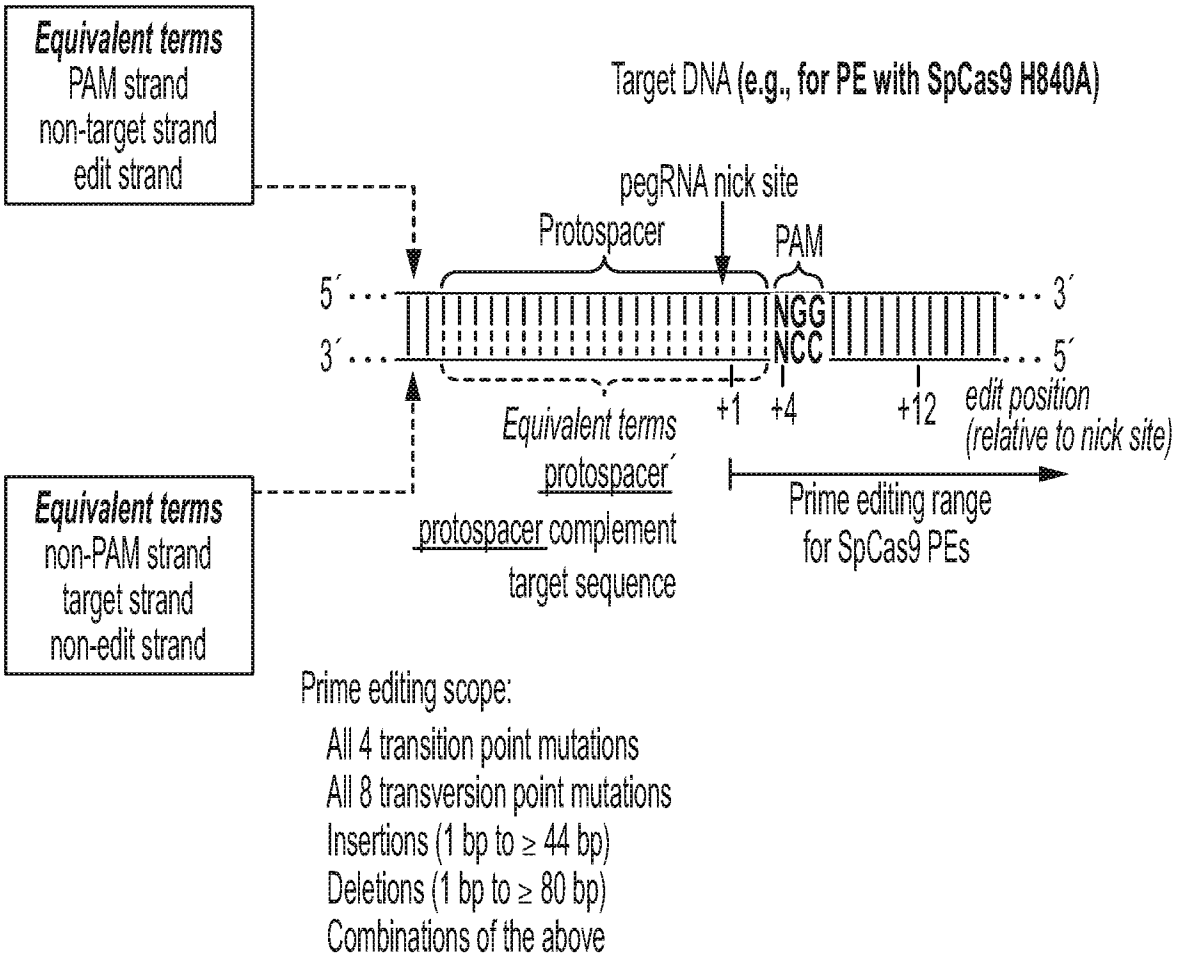

Equivalent terms
PAM strand
non-target strand
edit strand
Target DNA (e.g., for PE with SpCas9 H840A)
pegRNA nick site
Protospacer
PAM
5´
3´
NGG
NCC
3´
5´
+1
+4
+12
edit position
(relative to nick site)
Equivalent terms
protospacer
protospacer complement
target sequence
Prime editing range
for SpCas9 PEs
Equivalent terms
non-PAM strand
target strand
non-edit strand
Prime editing scope:
All 4 transition point mutations
All 8 transversion point mutations
Insertions (1 bp to ≥ 44 bp)
Deletions (1 bp to ≥ 80 bp)
Combinations of the above

FIG. 1K

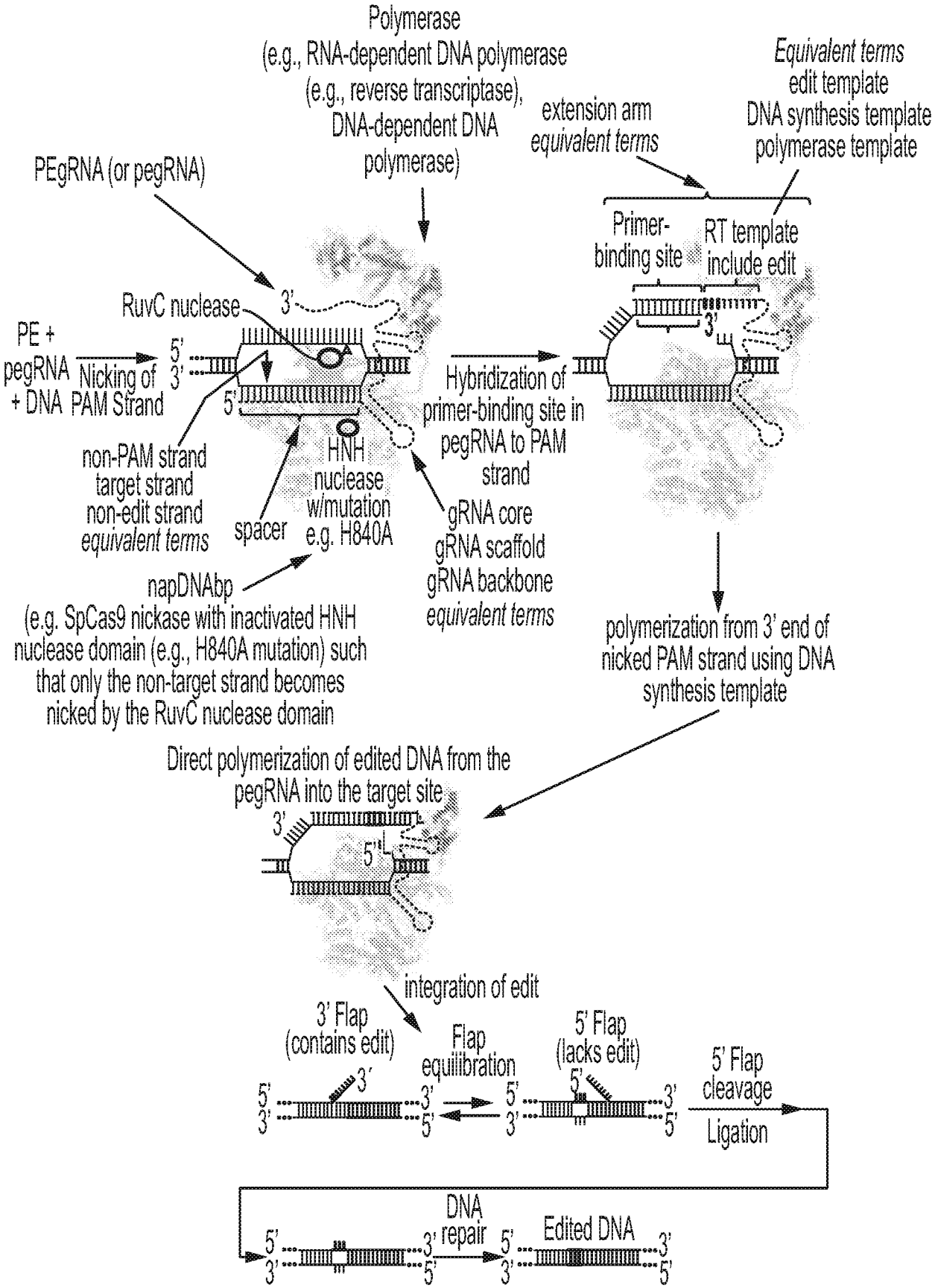

Polymerase
(e.g., RNA-dependent DNA polymerase
(e.g., reverse transcriptase),
DNA-dependent DNA
polymerase)
PEgRNA (or pegRNA)
extension arm
equivalent terms
Equivalent terms
edit template
DNA synthesis template
polymerase template
Primer-
binding site
RT template
include edit
RuvC nuclease
3'
PE +
pegRNA
+ DNA
Nicking of
PAM Strand
5'
3'
5'
Hybridization of
primer-binding site in
pegRNA to PAM
strand
non-PAM strand
target strand
non-edit strand
equivalent terms
spacer
HNH
nuclease
w/mutation
e.g. H840A
gRNA core
gRNA scaffold
gRNA backbone
equivalent terms
napDNAbp
(e.g. SpCas9 nickase with inactivated HNH
nuclease domain (e.g., H840A mutation) such
that only the non-target strand becomes
nicked by the RuvC nuclease domain
polymerization from 3' end of
nicked PAM strand using DNA
synthesis template
Direct polymerization of edited DNA from the
pegRNA into the target site
integration of edit
3' Flap
(contains edit)
Flap
equilibration
5' Flap
(lacks edit)
5' Flap
cleavage
Ligation
DNA
repair
Edited DNA

FIG. 1L

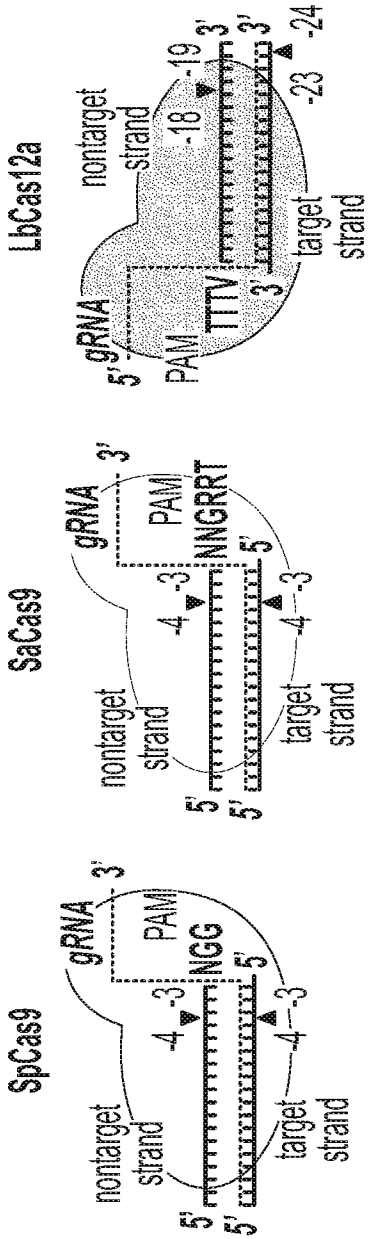

SpCas9
gRNA
3'
PAM
NGG
5'
-4
-3
nontarget strand
target strand
SaCas9
gRNA
3'
PAM
NNGRRT
5'
-4
-3
nontarget strand
target strand
LbCas12a
nontarget strand
5'
gRNA
PAM
TTTV
3'
-18
-19
-23
-24
target strand

FIG. 2

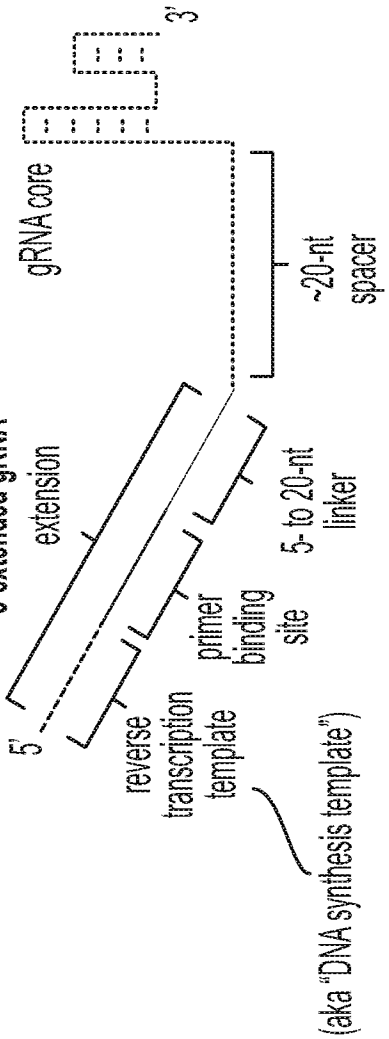

gRNA core
3'
~20-nt spacer
5' extended gRNA
extension
5'
reverse transcription template
primer binding site
5- to 20-nt linker
(aka "DNA synthesis template")

FIG. 3A

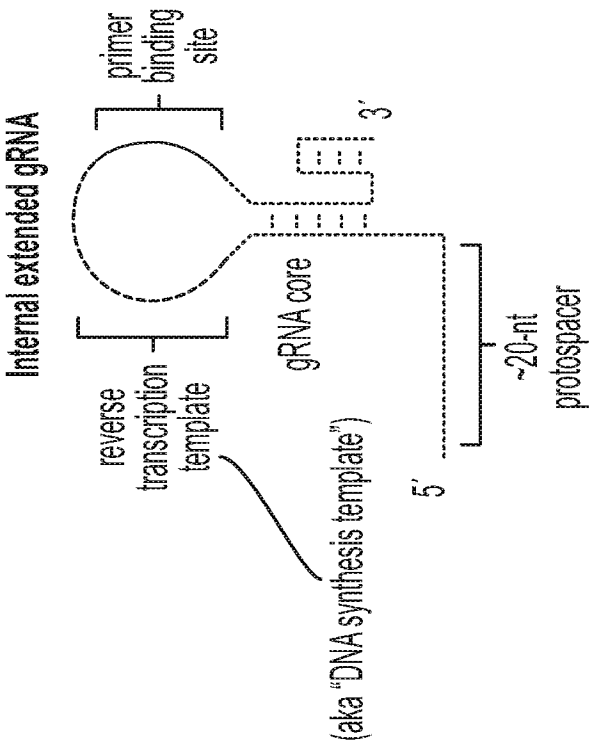

Internal extended gRNA
primer binding site
3'
gRNA core
reverse transcription template
(aka "DNA synthesis template")
5'
~20-nt protospacer

FIG. 3C

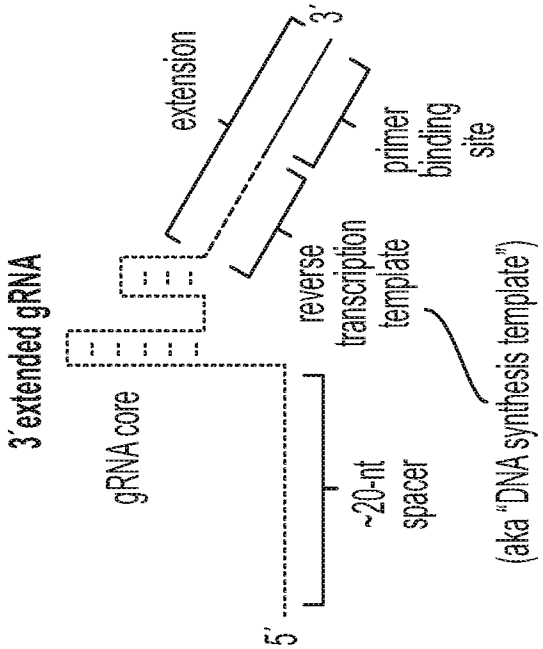

3' extended gRNA
extension
3'
gRNA core
reverse transcription template
primer binding site
5'
~20-nt spacer
(aka "DNA synthesis template")

FIG. 3B

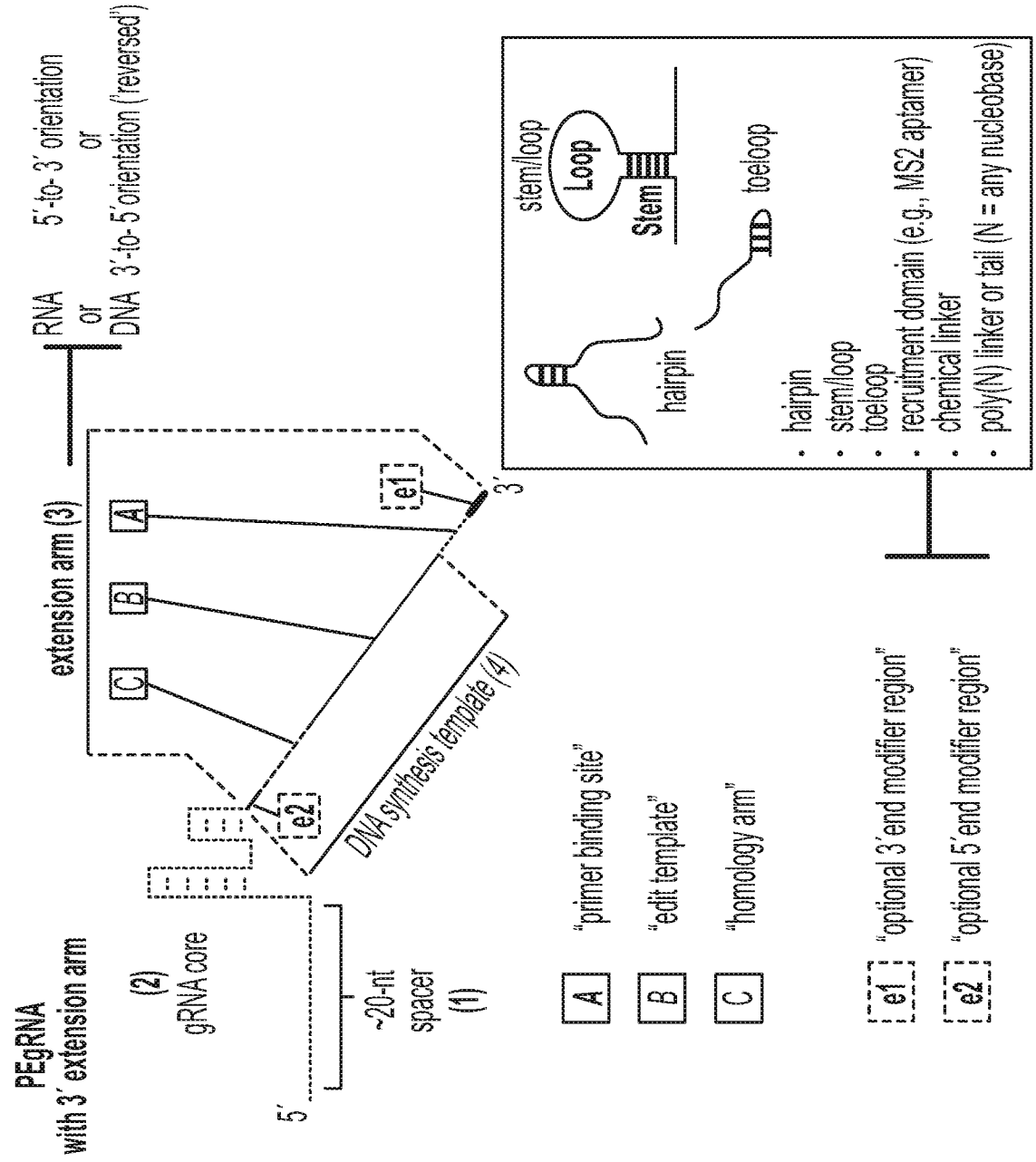

PEgRNA
with 3′ extension arm
(2)
gRNA core
~20-nt
spacer
(1)
5′
3′
extension arm (3)
RNA
or
DNA
5′-to-3′ orientation
or
3′-to-5′orientation ("reversed")
A
B
C
e1
e2
DNA synthesis template (4)
stem/loop
Loop
Stem
hairpin
toeloop
• hairpin
• stem/loop
• toeloop
• recruitment domain (e.g., MS2 aptamer)
• chemical linker
• poly(N) linker or tail (N = any nucleobase)
A "primer binding site"
B "edit template"
C "homology arm"
e1 "optional 3′end modifier region"
e2 "optional 5′end modifier region"

FIG. 3D

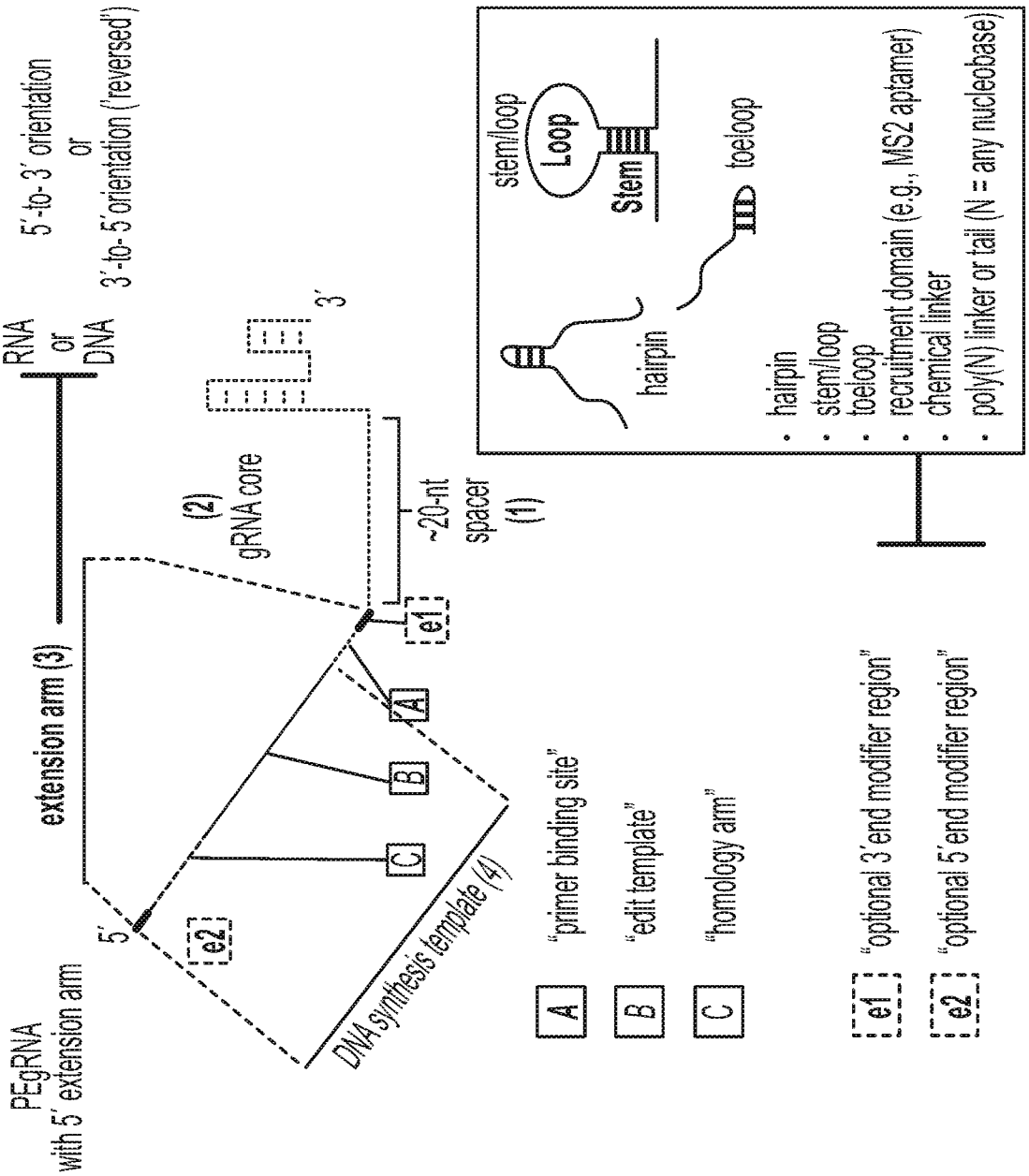

PEgRNA
with 5′ extension arm
5′
e2
DNA synthesis template (4)
C
B
A
e1
~20-nt
spacer
(1)
(2)
gRNA core
extension arm (3)
RNA
or
DNA
3′
5′-to- 3′ orientation
or
3′-to- 5′ orientation (“reversed”)
stem/loop
Loop
Stem
hairpin
toeloop
• hairpin
• stem/loop
• toeloop
• recruitment domain (e.g., MS2 aptamer)
• chemical linker
• poly(N) linker or tail (N = any nucleobase)
A “primer binding site”
B “edit template”
C “homology arm”
e1 “optional 3′ end modifier region”
e2 “optional 5′ end modifier region”

FIG. 3E

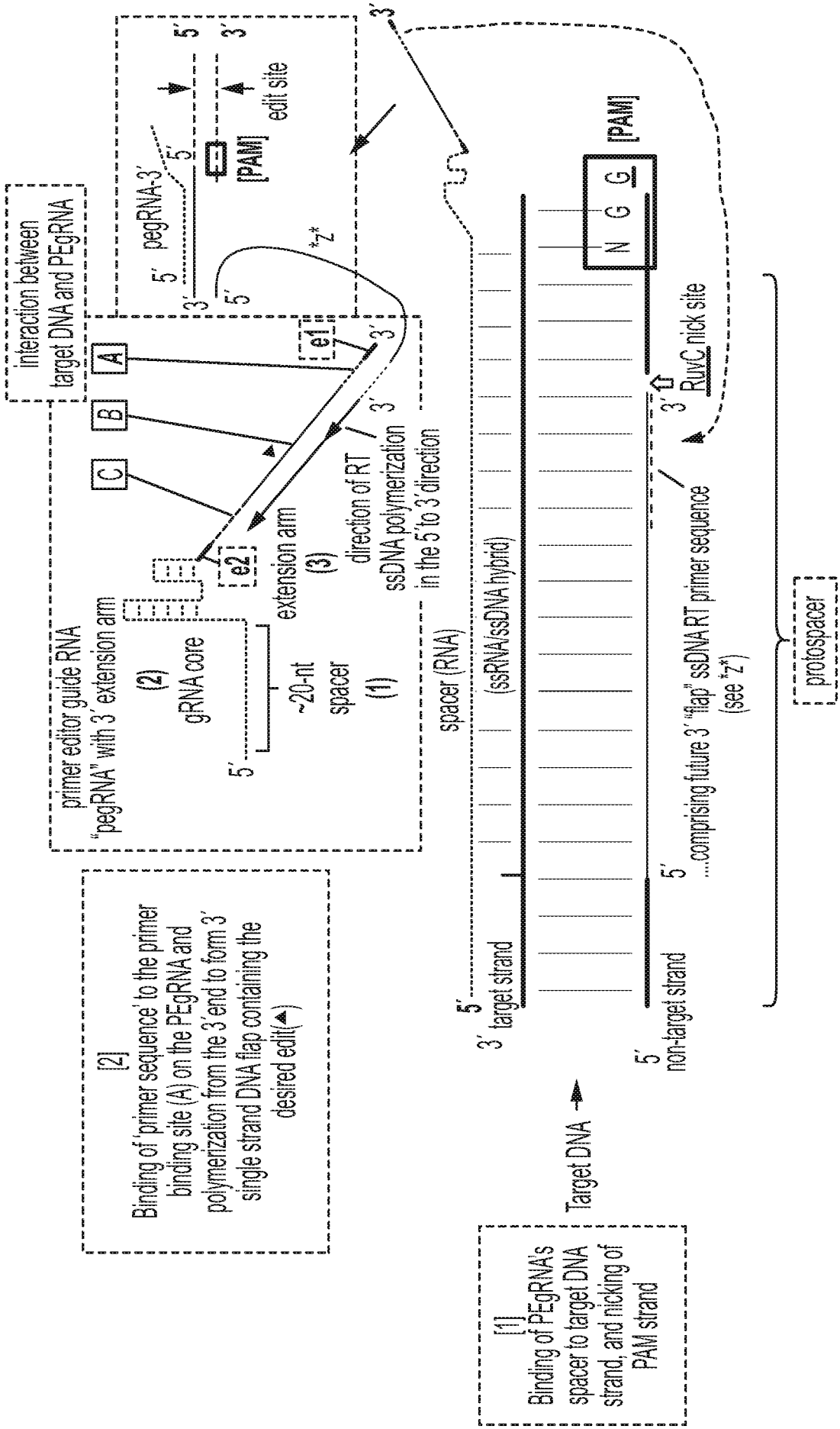

interaction between target DNA and PEgRNA
primer editor guide RNA "pegRNA" with 3′ extension arm
(2) gRNA core
~20-nt spacer (1)
extension arm (3)
direction of RT
ssDNA polymerization in the 5′ to 3′ direction
spacer (RNA)
A
B
C
e1
e2
pegRNA-3′
edit site
[PAM]
"z"
N G G
RuvC nick site
(ssRNA/ssDNA hybrid)
....comprising future 3′ "flap" ssDNA RT primer sequence (see "z")
protospacer
target strand
non-target strand
Target DNA
[1] Binding of PEgRNA's spacer to target DNA strand, and nicking of PAM strand
[2] Binding of 'primer sequence' to the primer binding site (A) on the PEgRNA and polymerization from the 3′ end to form 3′ single strand DNA flap containing the desired edit(▲)

FIG. 3F

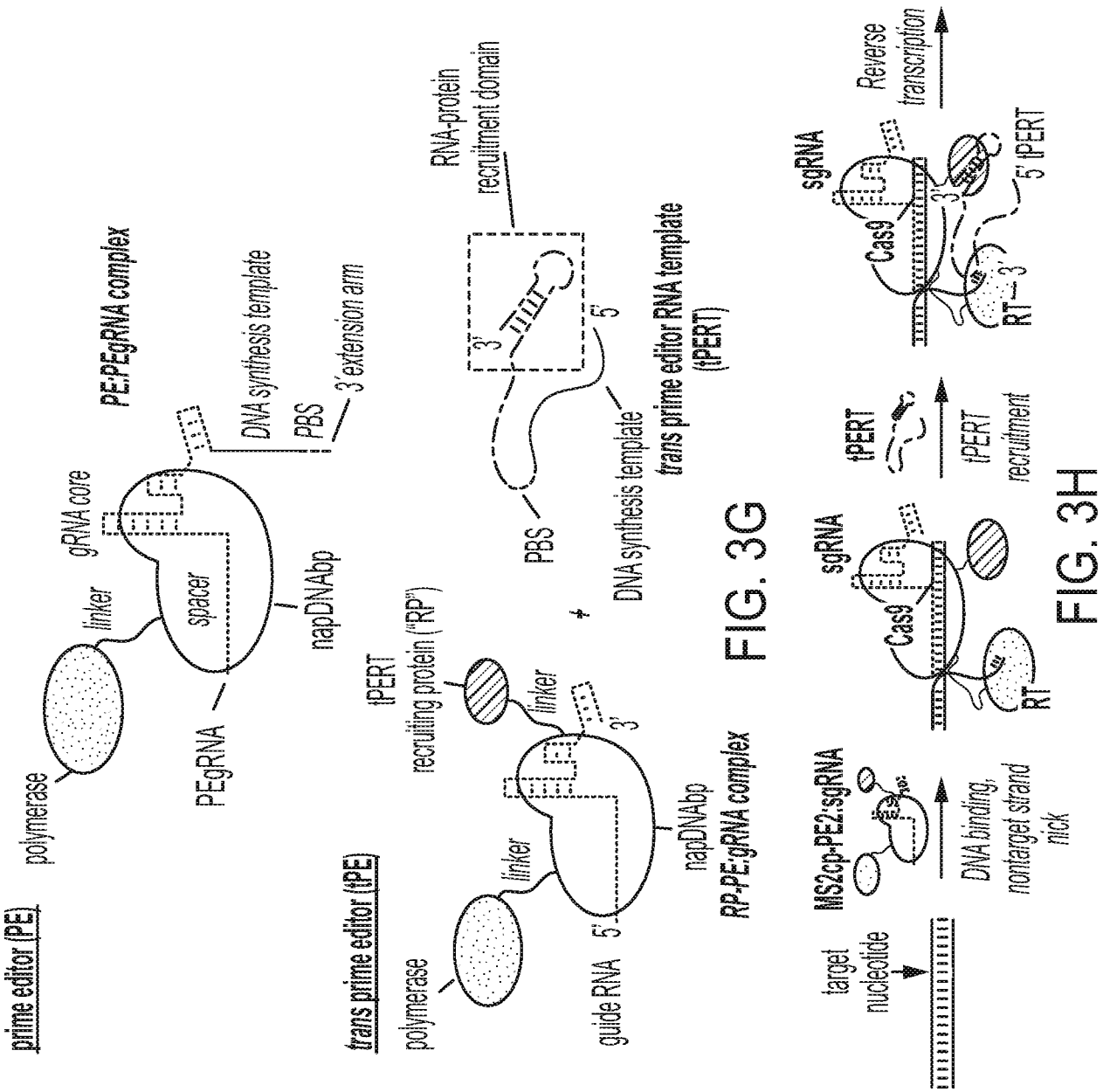

prime editor (PE)
polymerase
linker
gRNA core
spacer
napDNAbp
PE:PEgRNA complex
DNA synthesis template
PBS
3' extension arm
PEgRNA
trans prime editor (tPE)
polymerase
linker
guide RNA
5'
3'
napDNAbp
RP-PE:gRNA complex
tPERT
recruiting protein ("RP")
linker
RNA-protein
recruitment domain
3'
5'
PBS
DNA synthesis template
trans prime editor RNA template
(tPERT)
target
nucleotide
MS2cp-PE2:sgRNA
DNA binding,
nontarget strand
nick
sgRNA
Cas9
RT
tPERT
tPERT
recruitment
sgRNA
Cas9
RT
3'
5' tPERT
Reverse
transcription

FIG. 3G

FIG. 3H

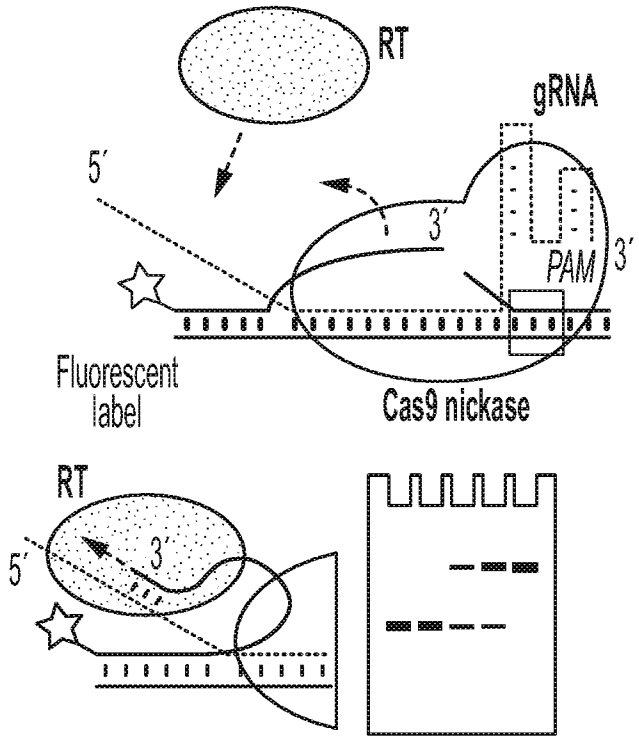


FIG. 4A

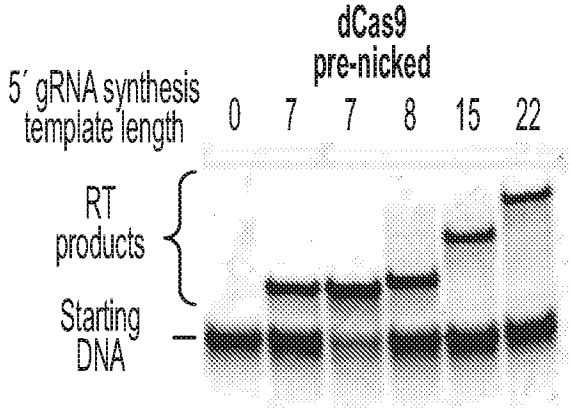


FIG. 4B

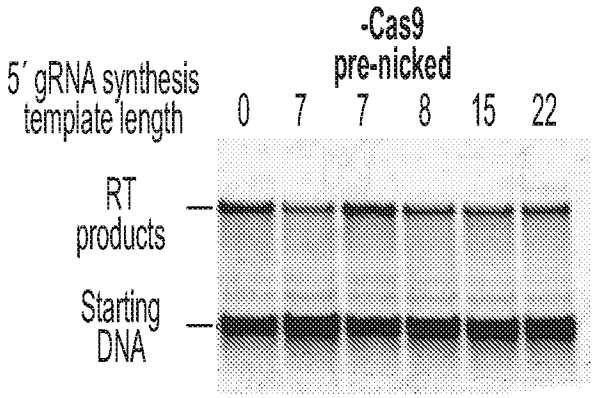


FIG. 4C

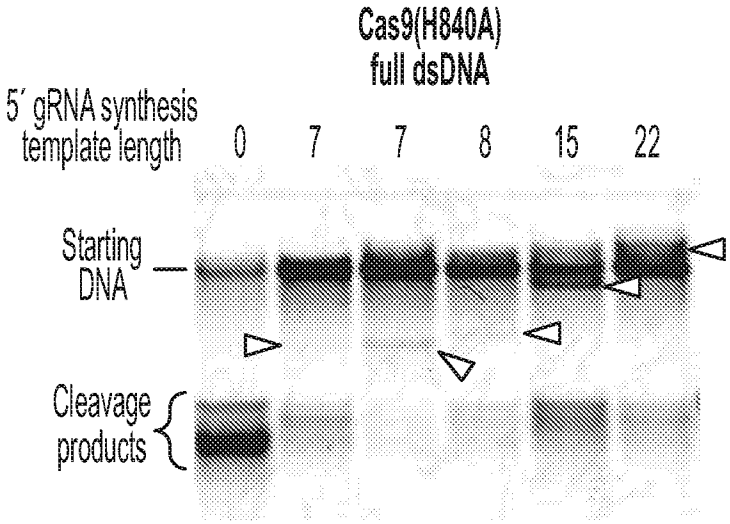

Cas9(H840A)
full dsDNA
5′ gRNA synthesis template length
0
7
7
8
15
22
Starting DNA
Cleavage products

FIG. 4D

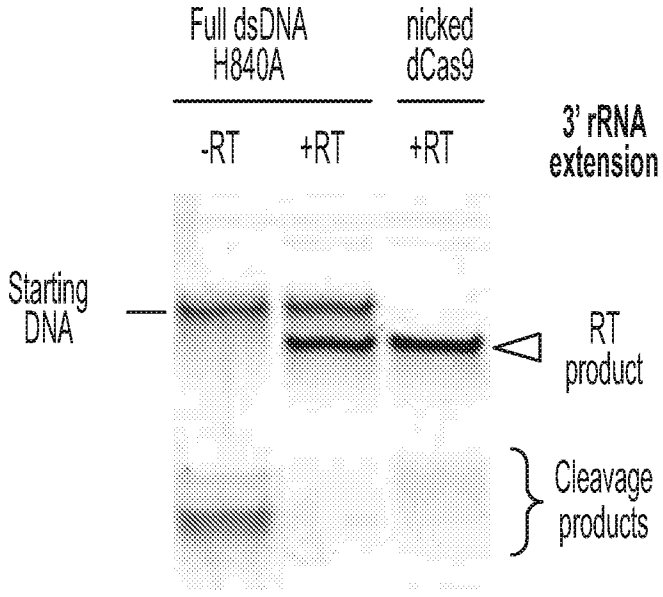

Full dsDNA H840A
nicked dCas9
-RT
+RT
+RT
3' rRNA extension
Starting DNA
RT product
Cleavage products

FIG. 4E

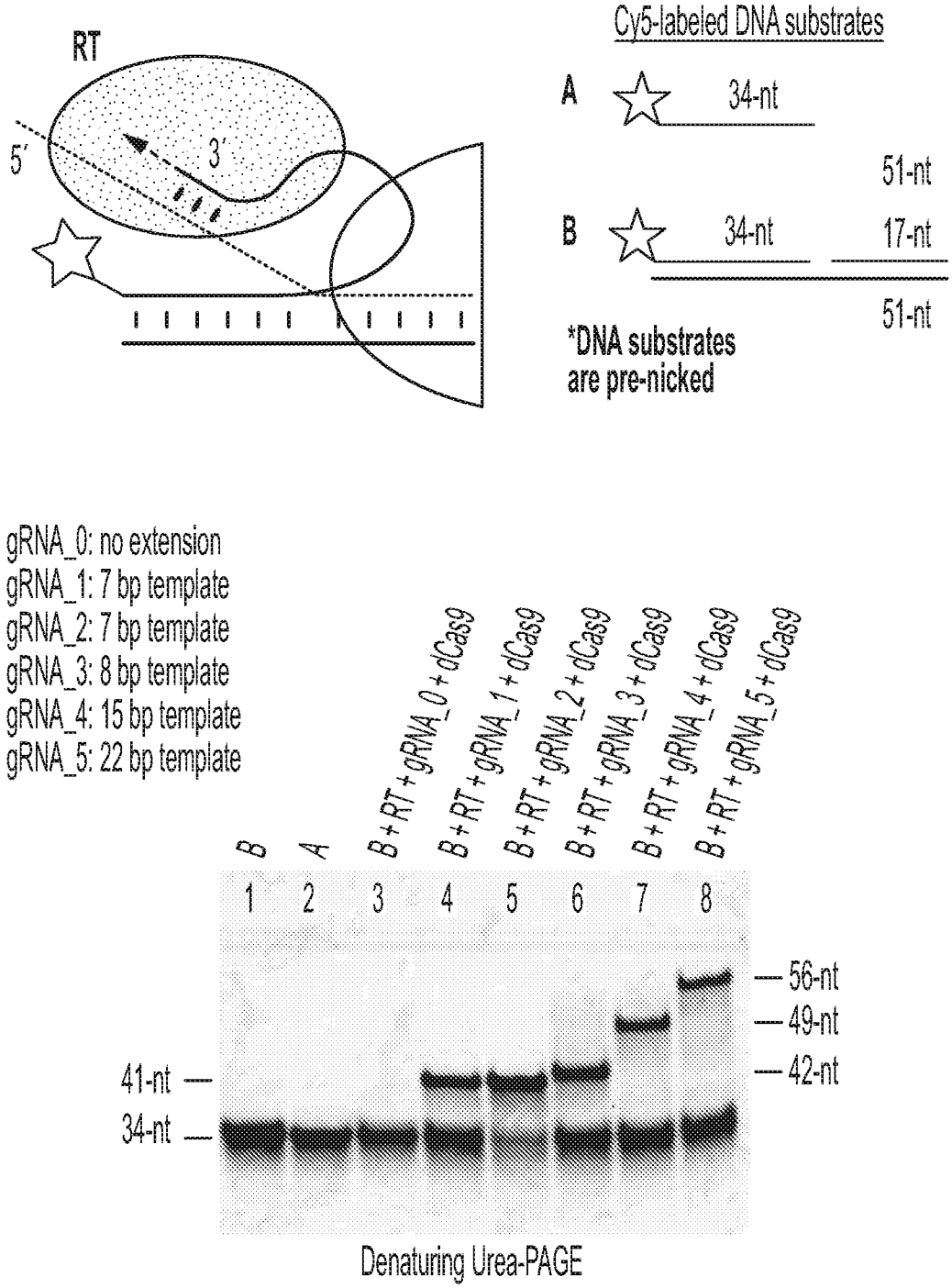

RT
5′
3′
Cy5-labeled DNA substrates
A
34-nt
51-nt
B
34-nt
17-nt
51-nt
*DNA substrates
are pre-nicked
gRNA_0: no extension
gRNA_1: 7 bp template
gRNA_2: 7 bp template
gRNA_3: 8 bp template
gRNA_4: 15 bp template
gRNA_5: 22 bp template
B
A
B+RT+gRNA_0+dCas9
B+RT+gRNA_1+dCas9
B+RT+gRNA_2+dCas9
B+RT+gRNA_3+dCas9
B+RT+gRNA_4+dCas9
B+RT+gRNA_5+dCas9
1 2 3 4 5 6 7 8
56-nt
49-nt
42-nt
41-nt
34-nt
Denaturing Urea-PAGE

FIG. 5

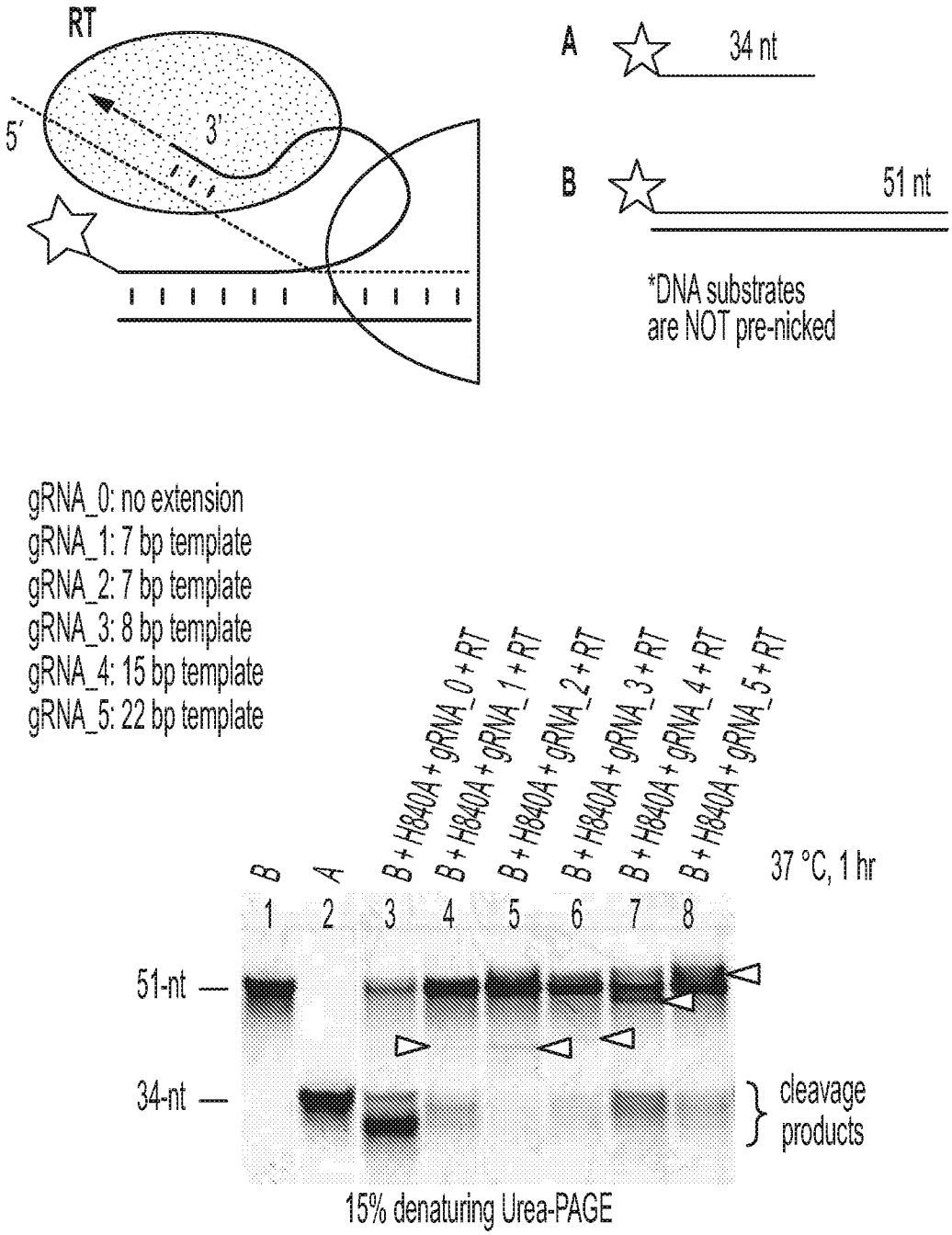

RT
5′
3′
A
34 nt
B
51 nt
*DNA substrates
are NOT pre-nicked
gRNA_0: no extension
gRNA_1: 7 bp template
gRNA_2: 7 bp template
gRNA_3: 8 bp template
gRNA_4: 15 bp template
gRNA_5: 22 bp template
B
A
B + H840A + gRNA_0 + RT
B + H840A + gRNA_1 + RT
B + H840A + gRNA_2 + RT
B + H840A + gRNA_3 + RT
B + H840A + gRNA_4 + RT
B + H840A + gRNA_5 + RT
37 °C, 1 hr
1 2 3 4 5 6 7 8
51-nt
34-nt
cleavage products
15% denaturing Urea-PAGE

FIG. 6

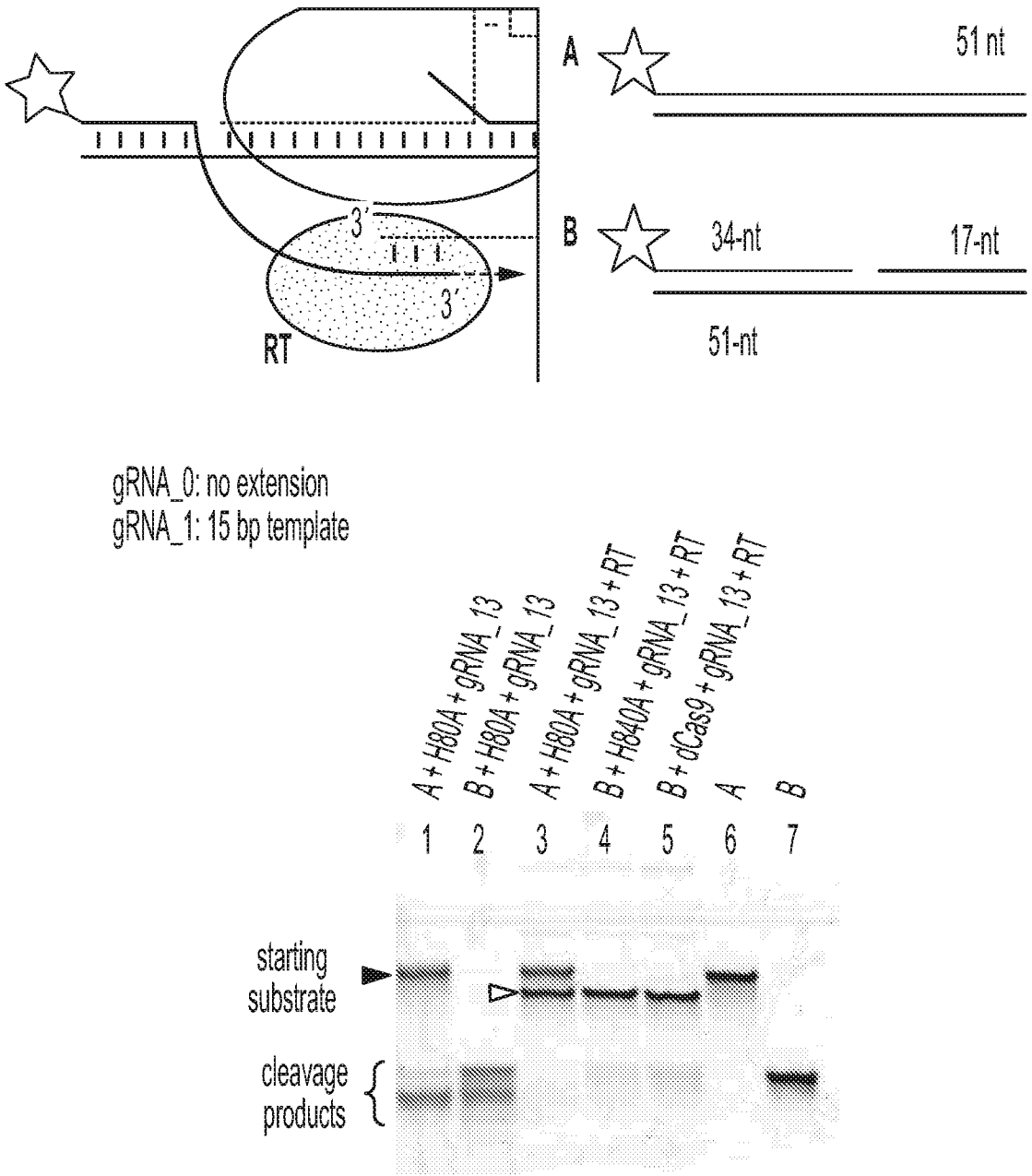

A
51 nt
B
34-nt
17-nt
51-nt
3′
3′
RT
gRNA_0: no extension
gRNA_1: 15 bp template
A + H80A + gRNA_13
B + H80A + gRNA_13
A + H80A + gRNA_13 + RT
B + H840A + gRNA_13 + RT
B + dCas9 + gRNA_13 + RT
A
B
1 2 3 4 5 6 7
starting substrate
cleavage products

FIG. 7

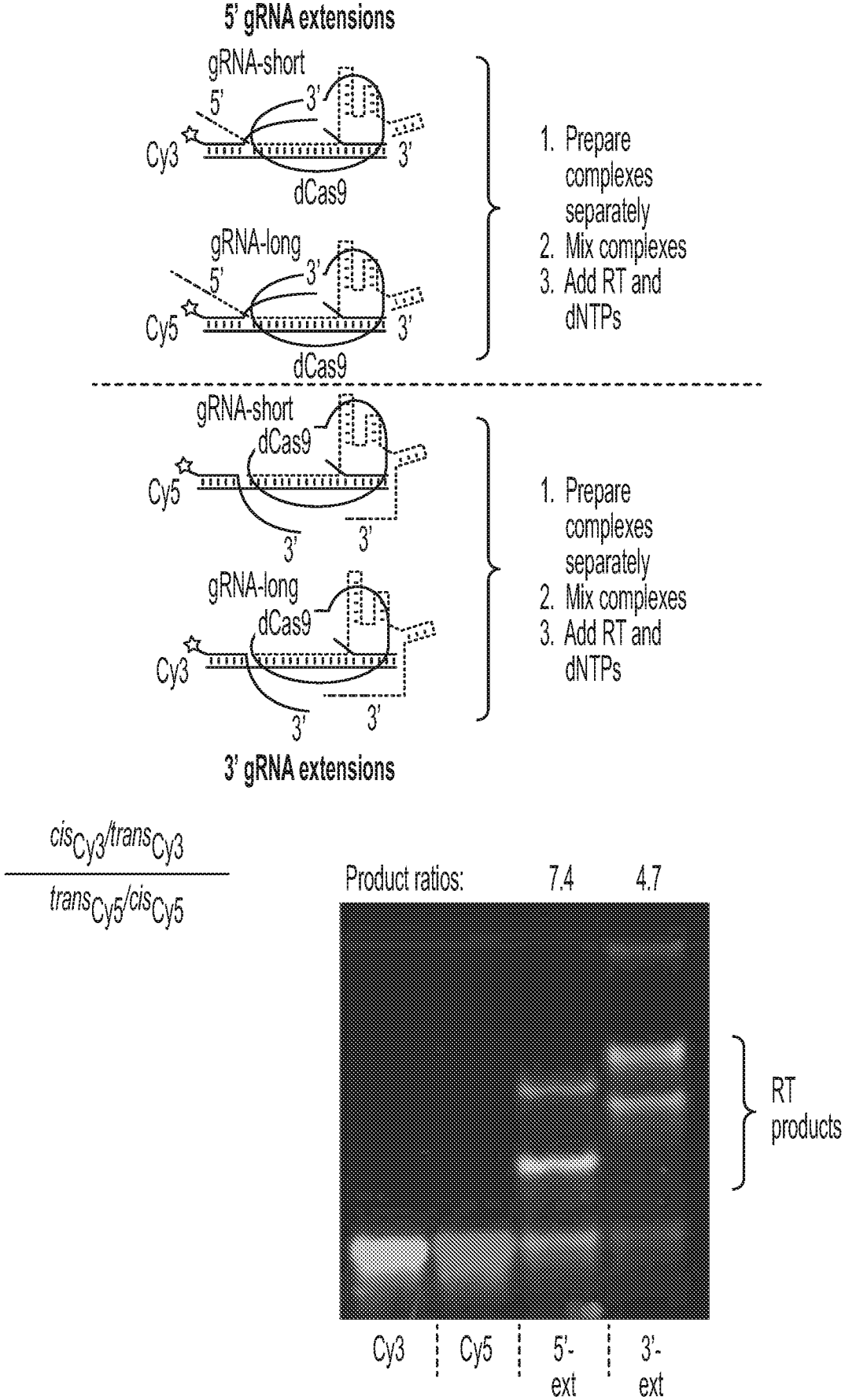

5' gRNA extensions
gRNA-short
5'
3'
Cy3
3'
dCas9
gRNA-long
5'
3'
Cy5
3'
dCas9
1. Prepare complexes separately
2. Mix complexes
3. Add RT and dNTPs
gRNA-short
dCas9
Cy5
3'
3'
gRNA-long
dCas9
Cy3
3'
3'
1. Prepare complexes separately
2. Mix complexes
3. Add RT and dNTPs
3' gRNA extensions
cisCy3/transCy3
transCy5/cisCy5
Product ratios:
7.4
4.7
RT products
Cy3
Cy5
5'-ext
3'-ext

FIG. 8

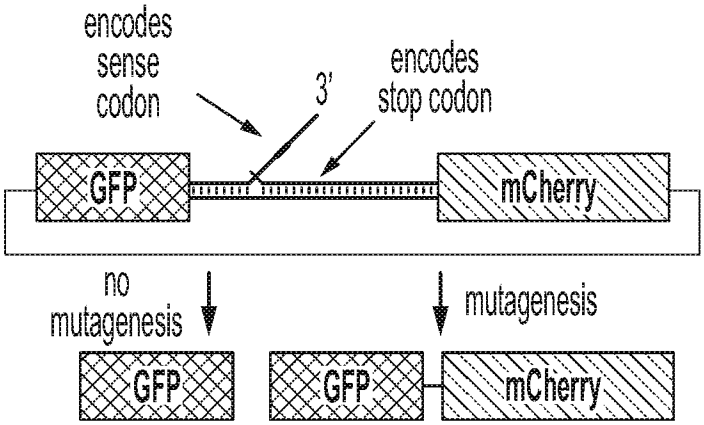

encodes
sense
codon
3'
encodes
stop codon
GFP
mCherry
no
mutagenesis
mutagenesis
GFP
GFP
mCherry

FIG. 9A

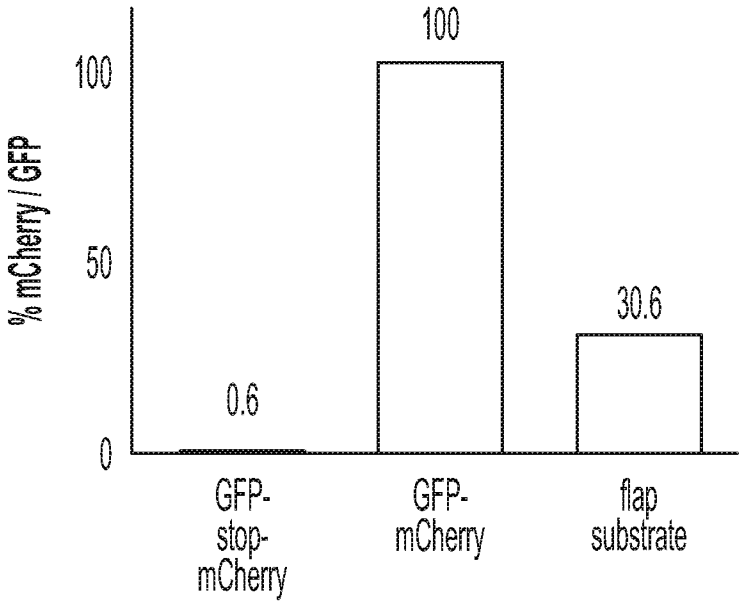

% mCherry / GFP
100
50
0
100
0.6
30.6
GFP-
stop-
mCherry
GFP-
mCherry
flap
substrate

FIG. 9B

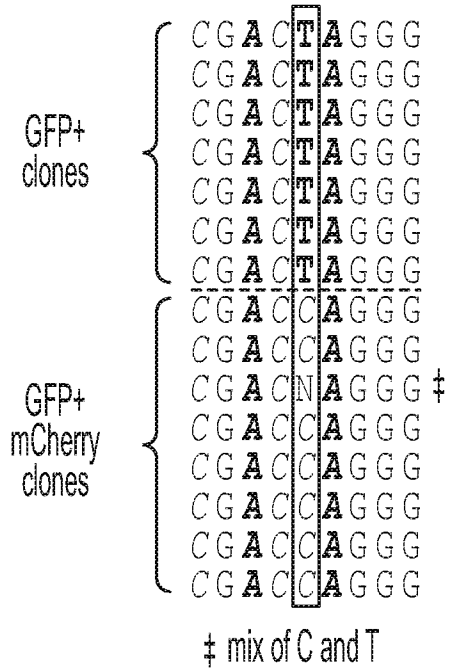

GFP+
clones
CGACTAGGG
CGACTAGGG
CGACTAGGG
CGACTAGGG
CGACTAGGG
CGACTAGGG
CGACTAGGG
GFP+
mCherry
clones
CGACCAGGG
CGACCAGGG
CGACNAGGG ‡
CGACCAGGG
CGACCAGGG
CGACCAGGG
CGACCAGGG
CGACCAGGG
‡ mix of C and T

FIG. 9C

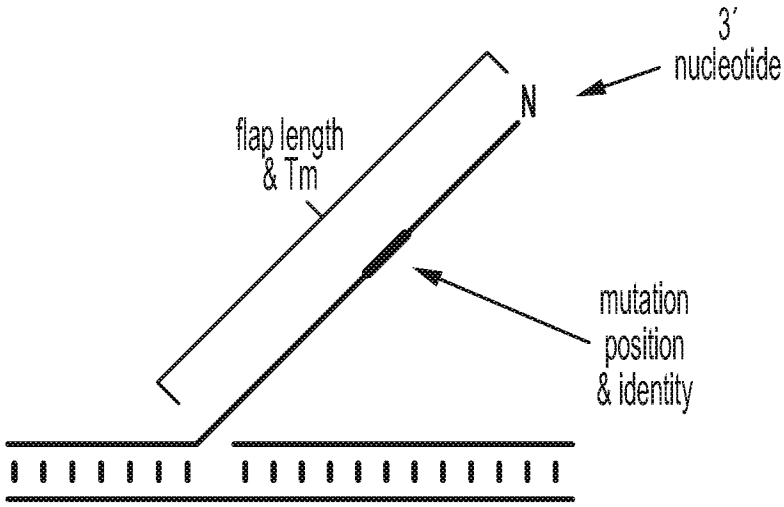

3′
nucleotide
N
flap length
& Tm
mutation
position
& identity

FIG. 9D

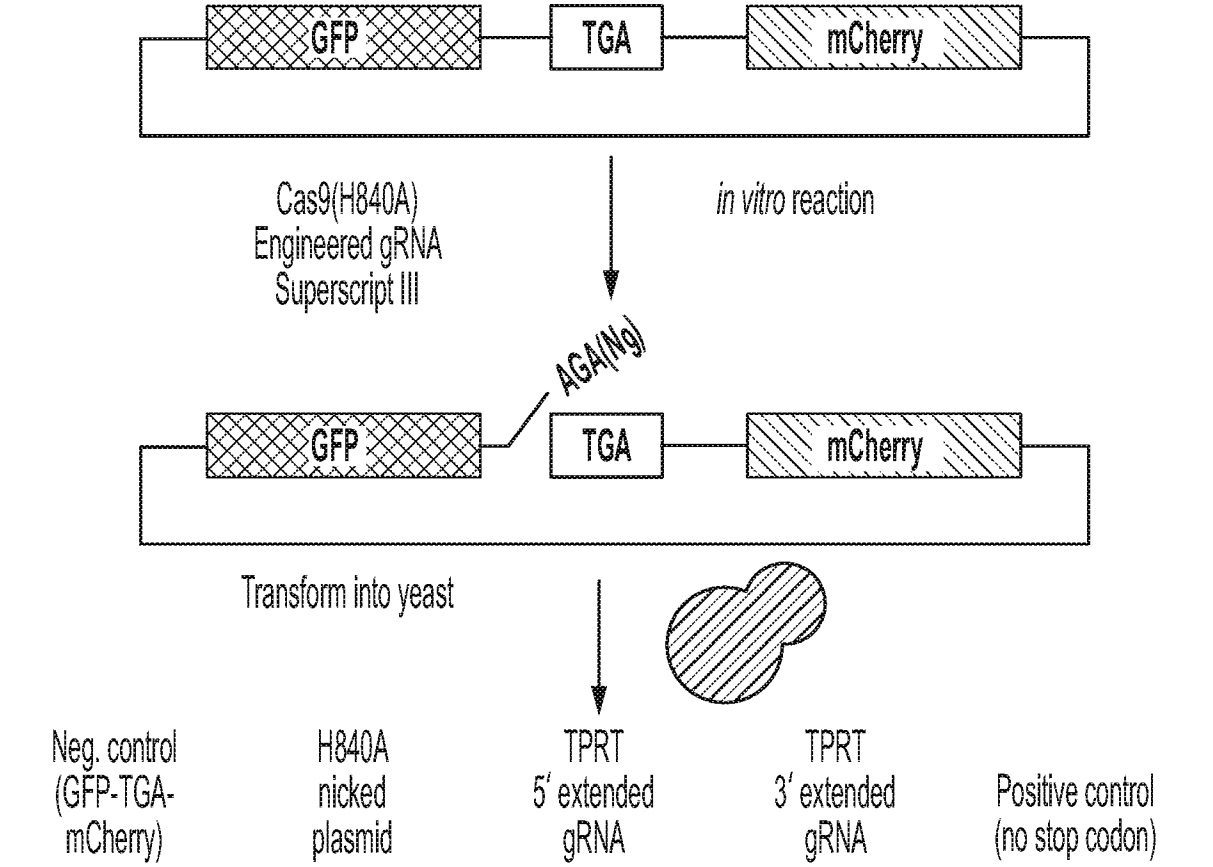

GFP
TGA
mCherry
Cas9(H840A)
Engineered gRNA
Superscript III
in vitro reaction
AGA(Ng)
GFP
TGA
mCherry
Transform into yeast
Neg. control (GFP-TGA-mCherry)
H840A nicked plasmid
TPRT 5′ extended gRNA
TPRT 3′ extended gRNA
Positive control (no stop codon)

FIG. 10

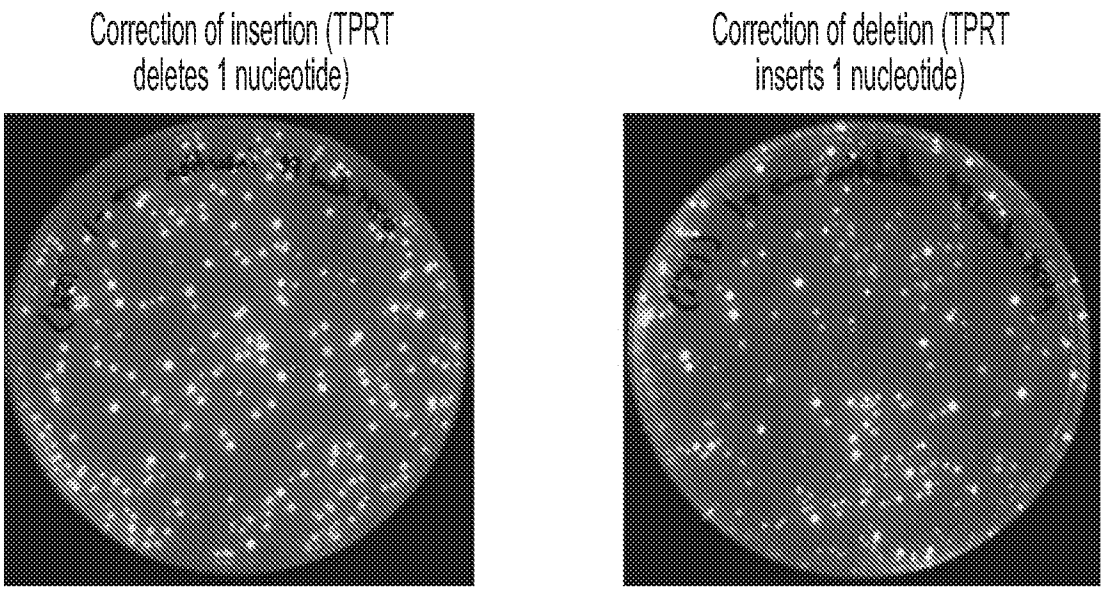

Correction of insertion (TPRT deletes 1 nucleotide)
Correction of deletion (TPRT inserts 1 nucleotide)

FIG. 11

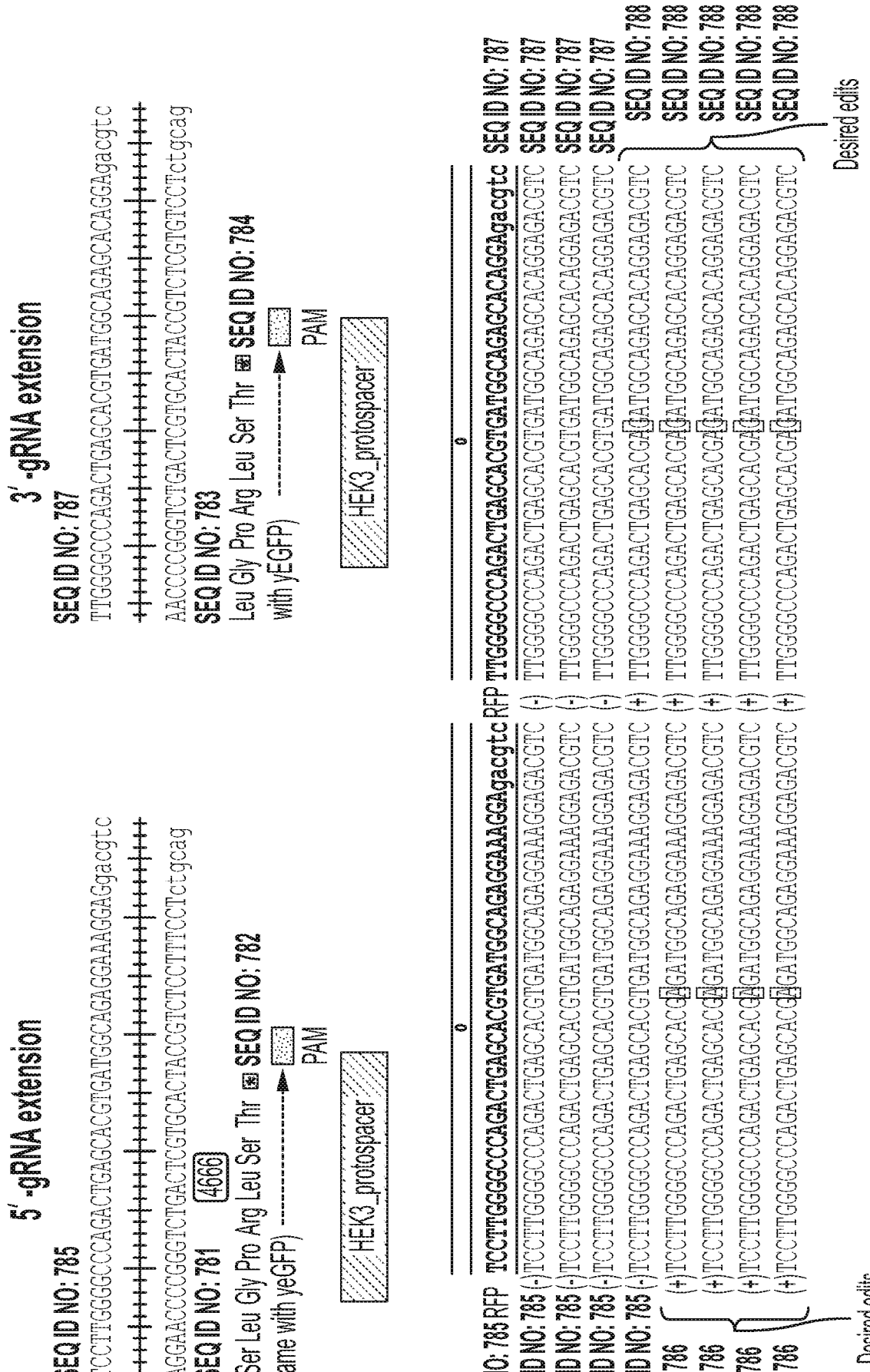

5′ -gRNA extension
SEQ ID NO: 785
TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGgacgtc
AGGAACCCCGGGTCTGACTCGTGCACTACCGTCTCCTTTCCTctgcag
SEQ ID NO: 781
4666
Ser Leu Gly Pro Arg Leu Ser Thr
SEQ ID NO: 782
frame with yeGFP)
PAM
HEK3_protospacer
3′ -gRNA extension
SEQ ID NO: 787
TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAgacgtc
AACCCCGGGTCTGACTCGTGCACTACCGTCTCCGTGTCCTctgcag
SEQ ID NO: 783
Leu Gly Pro Arg Leu Ser Thr
SEQ ID NO: 784
with yEGFP)
PAM
HEK3_protospacer
SEQ ID NO: 785 RFP TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGgacgtc RFP TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAgacgtc SEQ ID NO: 787
SEQ ID NO: 785 (-) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (-) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 787
SEQ ID NO: 785 (-) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (-) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 787
SEQ ID NO: 785 (-) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (-) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 787
SEQ ID NO: 785 (-) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (+) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 788
SEQ ID NO: 786 (+) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (+) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 788
SEQ ID NO: 786 (+) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (+) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 788
SEQ ID NO: 786 (+) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (+) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 788
SEQ ID NO: 786 (+) TCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAGACGTC (+) TTGGGGCCCAGACTGAGCACGTGATGGCAGAGGCACAGGAGACGTC SEQ ID NO: 788
Desired edits
Desired edits

FIG. 12

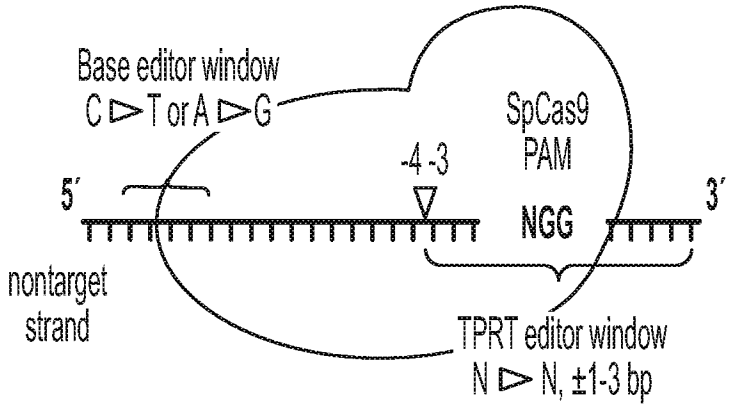

Base editor window
C ⊳ T or A ⊳ G
SpCas9
PAM
-4 -3
5′
3′
NGG
nontarget
strand
TPRT editor window
N ⊳ N, ±1-3 bp

FIG. 13

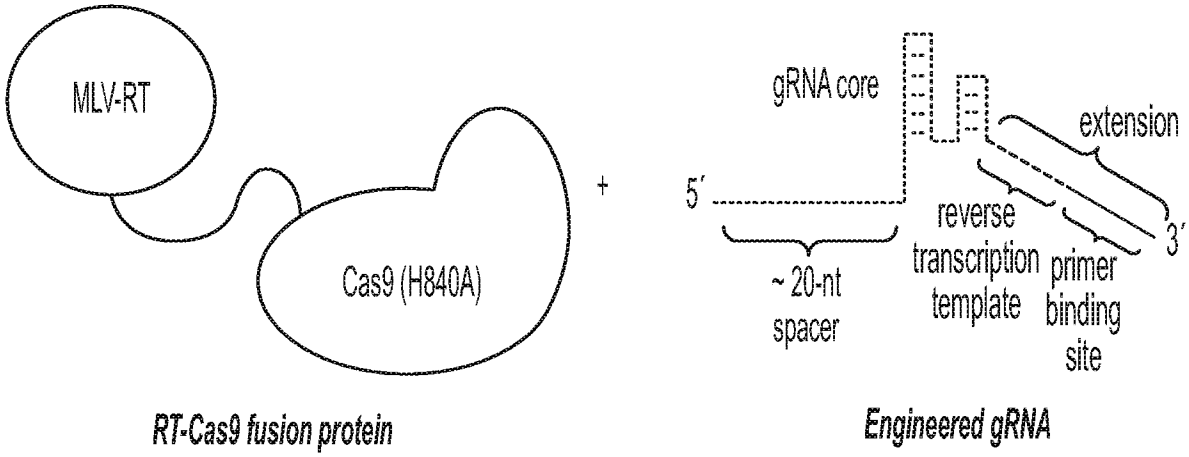

MLV-RT
Cas9 (H840A)
+
gRNA core
extension
5′
3′
reverse
transcription
template
primer
binding
site
~ 20-nt
spacer
RT-Cas9 fusion protein
Engineered gRNA

FIG. 14

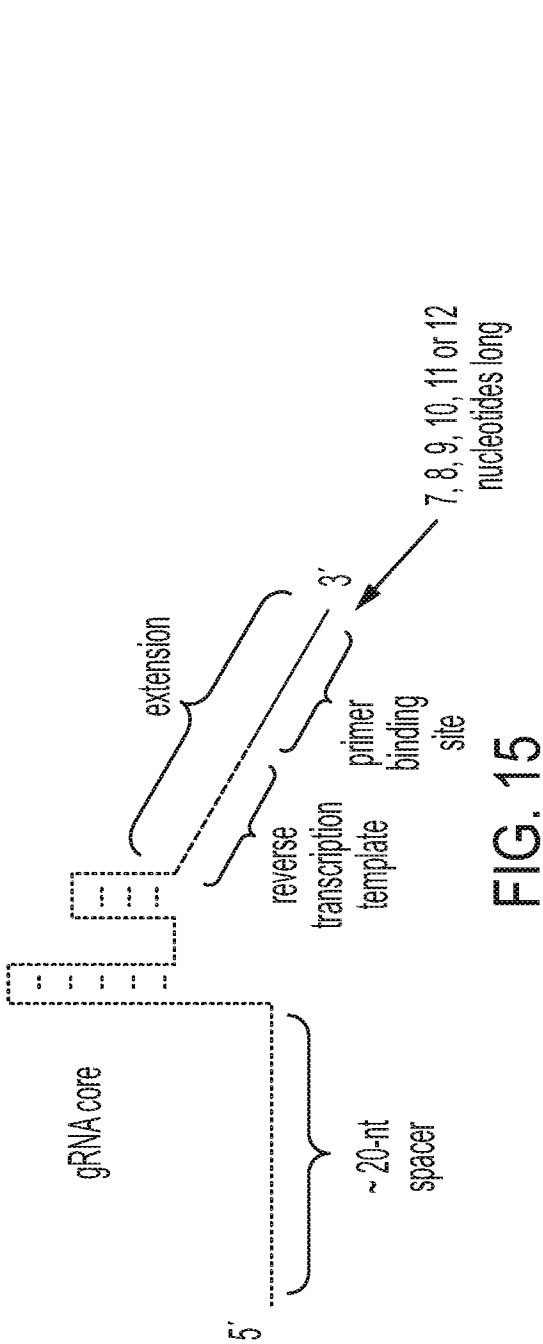

gRNA core
~ 20-nt spacer
5'
reverse transcription template
primer binding site
3'
extension
7, 8, 9, 10, 11 or 12 nucleotides long

FIG. 15

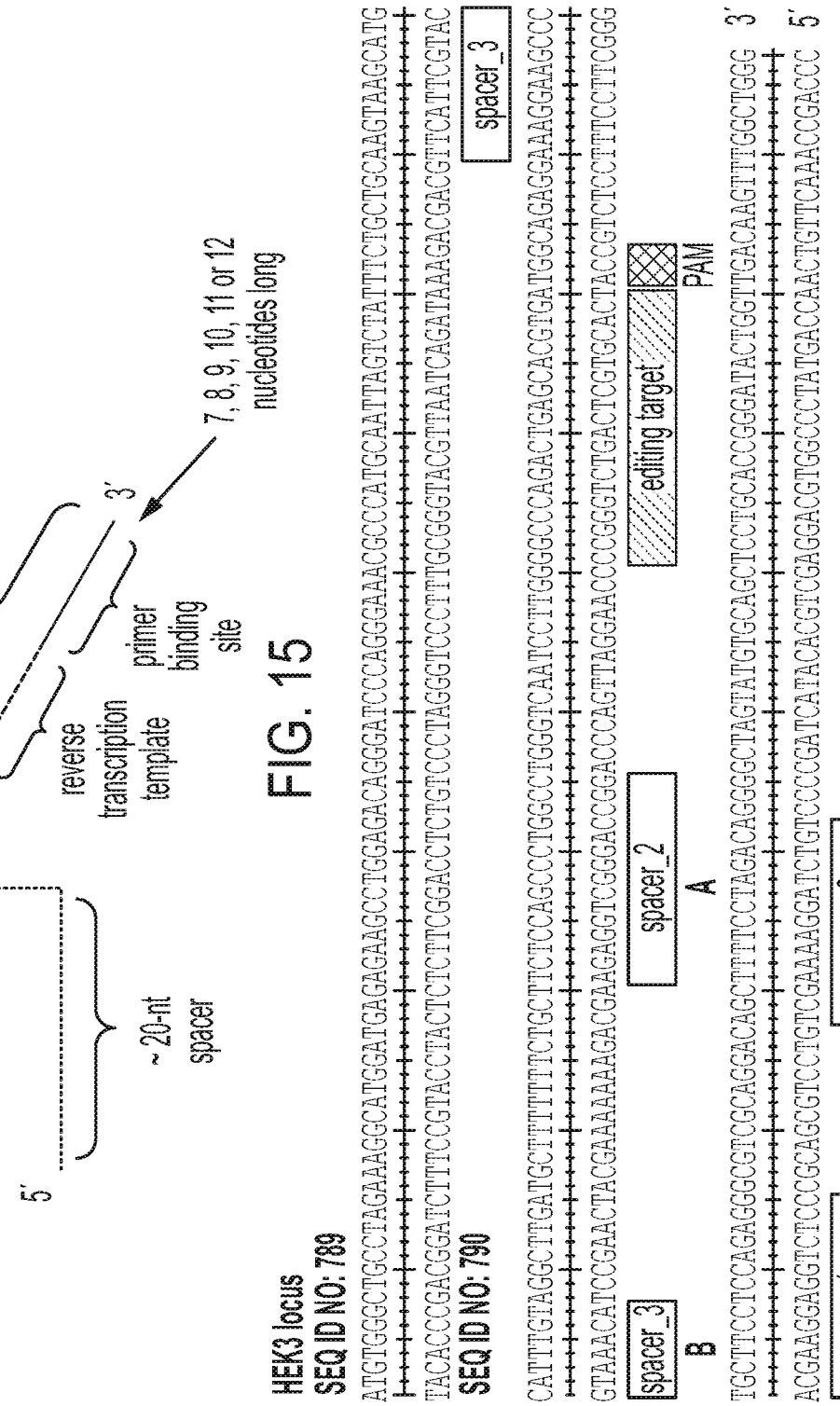

HEK3 locus
SEQ ID NO: 789
5' ATGTGGGCTGCCTAGAAAGGCATGGATGAGACAGGGATCCCAGGGAAACGCCCATGCAATTAGTCTATTTCTGCTGCAAGTAAGCATG
3' TACACCCGACGGATCTTTCCGTACCTACTCTCTTCGGACCTCTGTCCCTAGGGTCCCTTTGCGGGTACGTTAATCAGATAAAGACGACGTTCATTCGTAC
SEQ ID NO: 790
spacer_3
CATTTGTAGGCTTGATGCTTTTTTTTCTGCTTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAGCCC
GTAAACATCCGAACTACGAAAAAAGACGAAGAGGTCGGGACCGGACCCAGTTAGGAACCCCGGGTCTGACTCGTGCACTACCGTCTCCTTTCCTTCGGG
spacer_3
B
spacer_2
A
editing target
PAM
TGCTTCCTCCAGAGGGCGTCGCAGGACACAGCTTTTCCTAGACAGGGGCTAGTATGTGCAGCTCCTGCACCGGGATACTGGTTGACAAGTTTGGCTGGG 3'
ACGAAGGAGGTCTCCCCGCAGCGTCCTGTCGAAAAGGATCTGTCCCCGATCATACACGTCGAGGACGTGGCCCTATGACCAACTGTTCAAACCGACCC 5'
297
spacer_4_rev
C
spacer_5_rev
D
spacer_6
E

FIG. 16

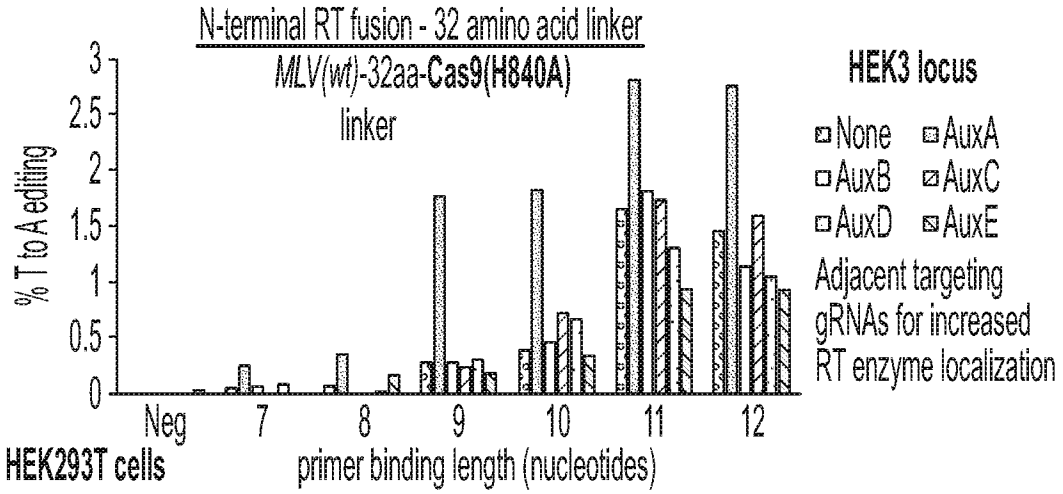


FIG. 17A

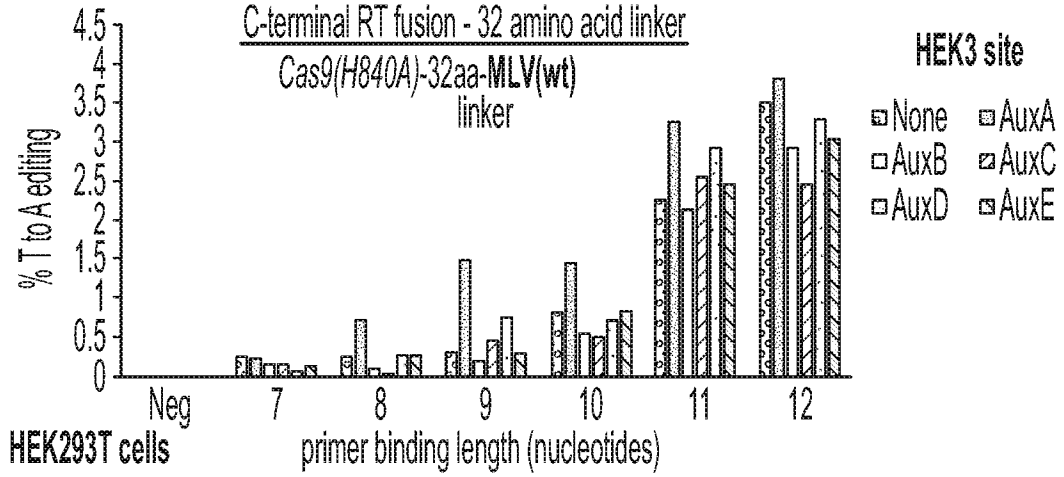


FIG. 17B

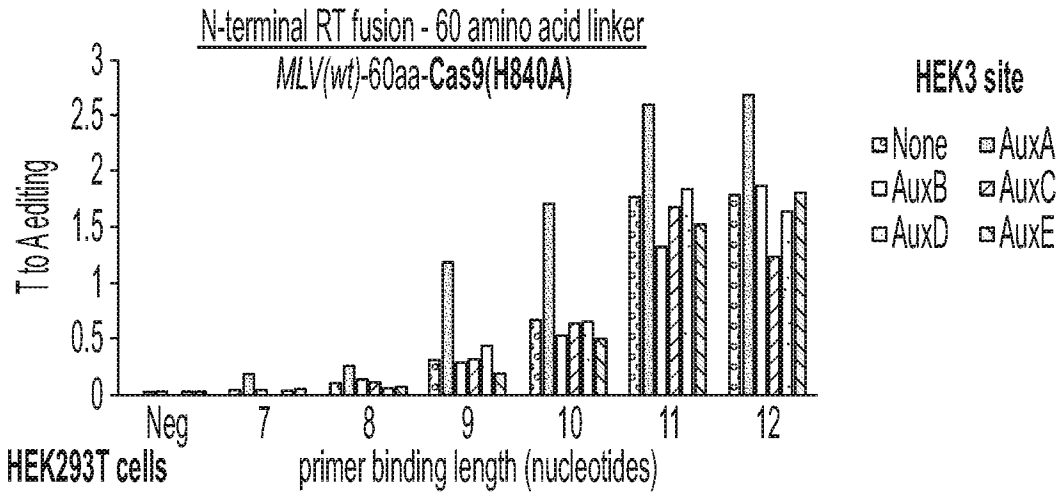


FIG. 17C

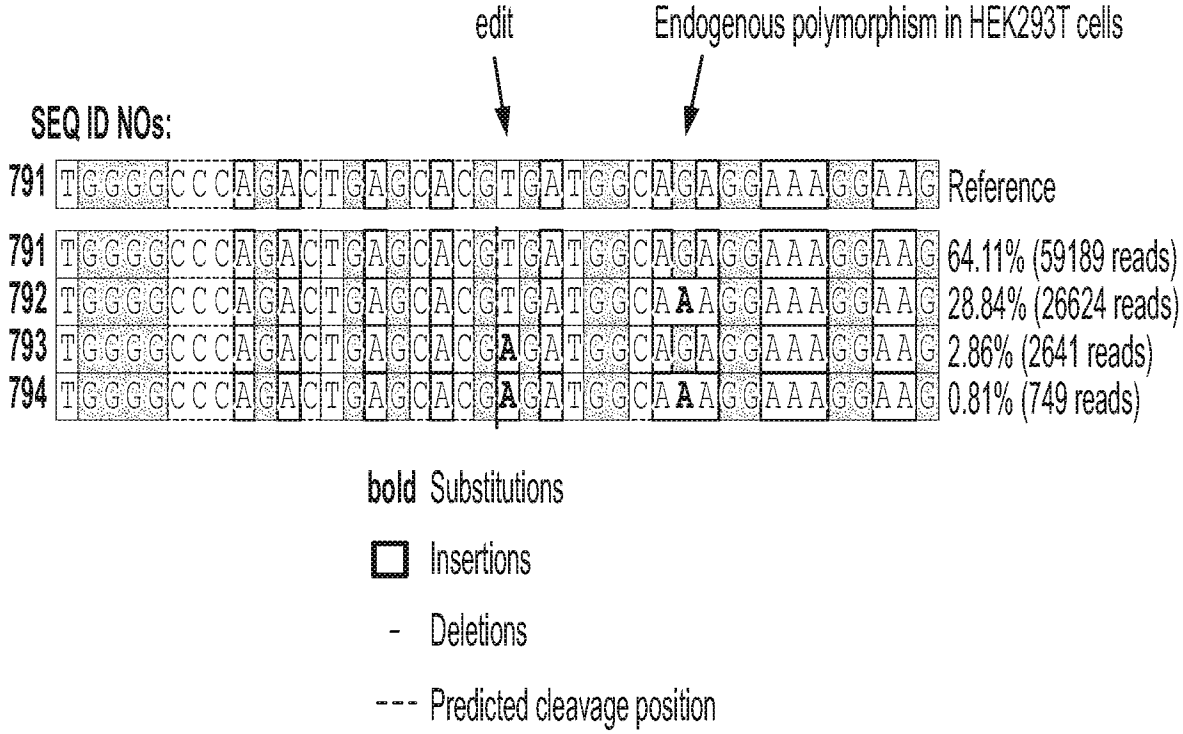

edit
Endogenous polymorphism in HEK293T cells
SEQ ID NOs:
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG Reference
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG 64.11% (59189 reads)
792 TGGGGCCCAGACTGAGCACGTGATGGCAAAGGAAAGGAAG 28.84% (26624 reads)
793 TGGGGCCCAGACTGAGCACGAGATGGCAGAGGAAAGGAAG 2.86% (2641 reads)
794 TGGGGCCCAGACTGAGCACGAGATGGCAAAGGAAAGGAAG 0.81% (749 reads)
bold Substitutions
Insertions
- Deletions
--- Predicted cleavage position

FIG. 18

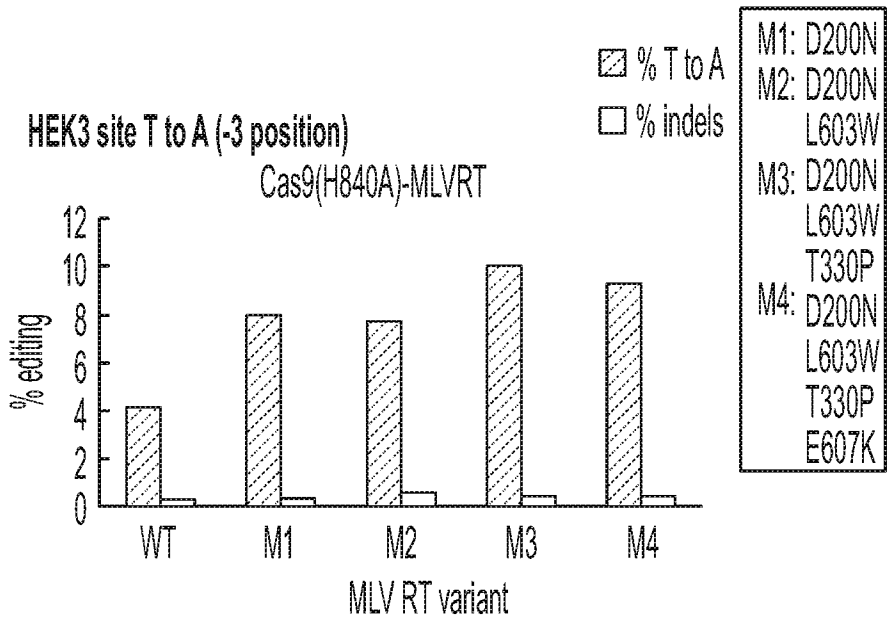

HEK3 site T to A (-3 position)
Cas9(H840A)-MLVRT
% T to A
% indels
% editing
12
10
8
6
4
2
0
WT
M1
M2
M3
M4
MLV RT variant
M1: D200N
M2: D200N L603W
M3: D200N L603W T330P
M4: D200N L603W T330P E607K

FIG. 19

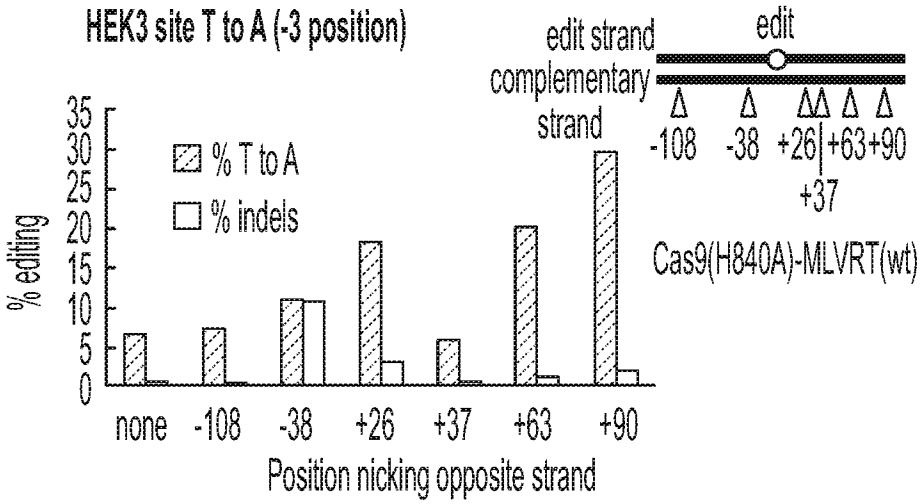

HEK3 site T to A (-3 position)
% T to A
% indels
% editing
35
30
25
20
15
10
5
0
none
-108
-38
+26
+37
+63
+90
Position nicking opposite strand
edit strand
complementary strand
edit
-108
-38
+26
+37
+63
+90
Cas9(H840A)-MLVRT(wt)

FIG. 20

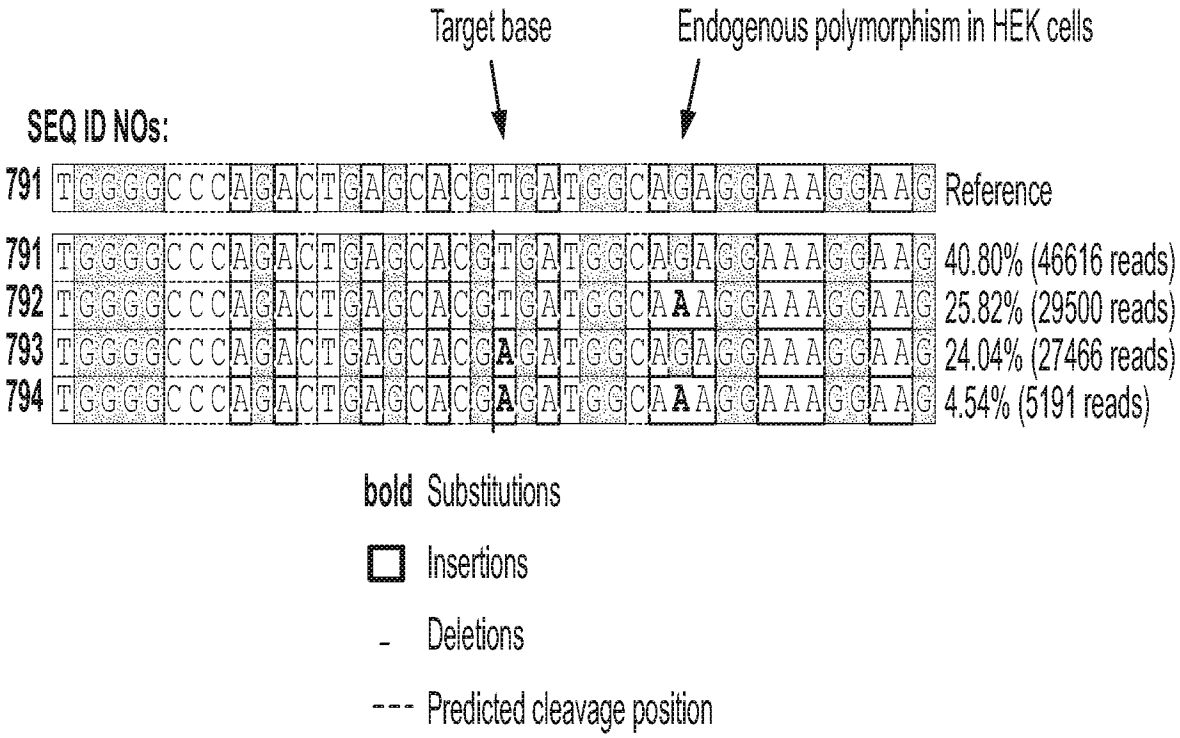

Target base
Endogenous polymorphism in HEK cells
SEQ ID NOs:
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG Reference
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG 40.80% (46616 reads)
792 TGGGGCCCAGACTGAGCACGTGATGGCAAAGGAAAGGAAG 25.82% (29500 reads)
793 TGGGGCCCAGACTGAGCACGAGATGGCAGAGGAAAGGAAG 24.04% (27466 reads)
794 TGGGGCCCAGACTGAGCACGAGATGGCAAAGGAAAGGAAG 4.54% (5191 reads)
bold Substitutions
Insertions
- Deletions
--- Predicted cleavage position

FIG. 21

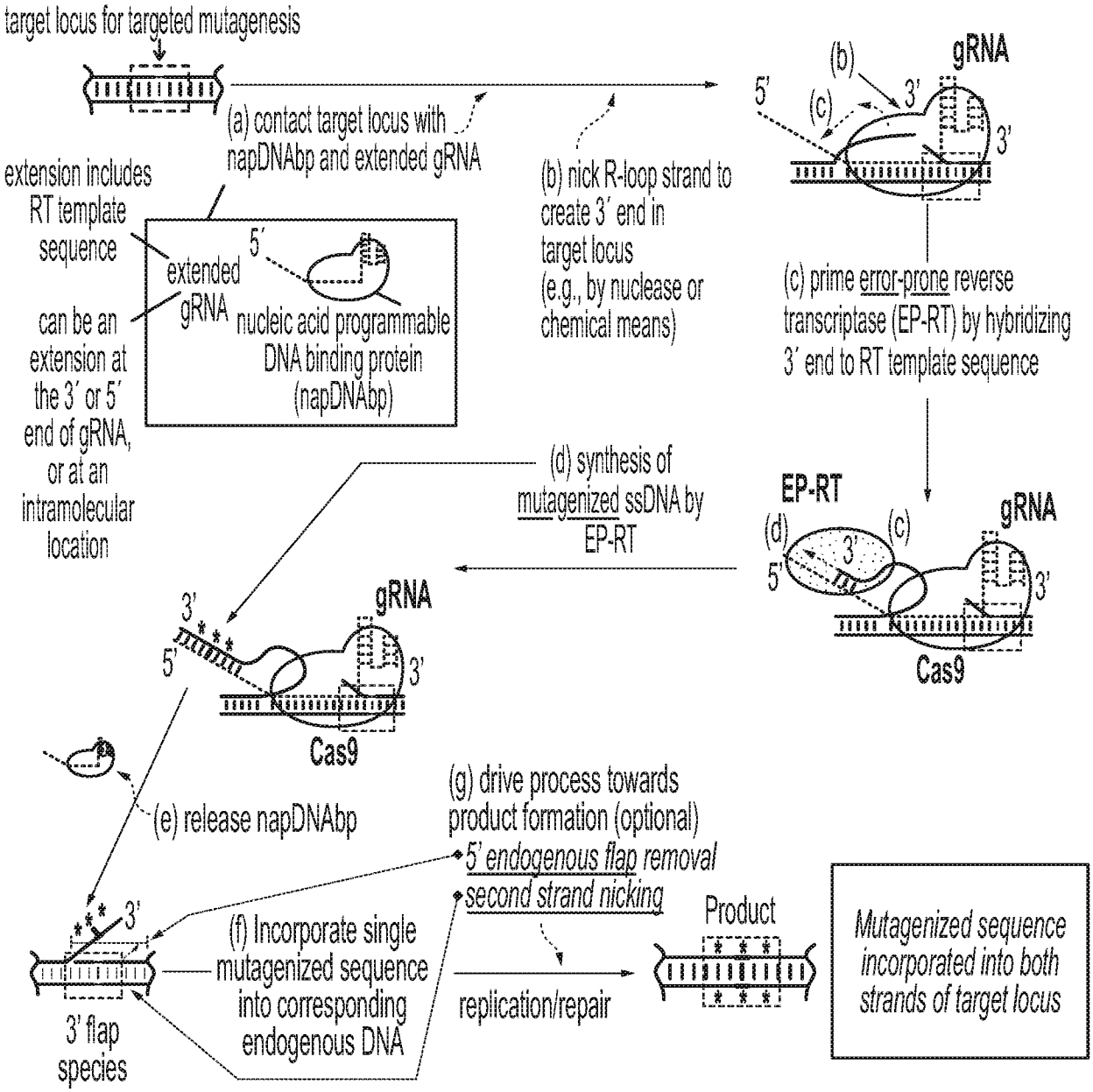

target locus for targeted mutagenesis
(a) contact target locus with napDNAbp and extended gRNA
extension includes RT template sequence
can be an extension at the 3′ or 5′ end of gRNA, or at an intramolecular location
extended gRNA
5′
nucleic acid programmable DNA binding protein (napDNAbp)
(b) nick R-loop strand to create 3′ end in target locus (e.g., by nuclease or chemical means)
(b)
gRNA
5′
(c)
3′
3′
(c) prime error-prone reverse transcriptase (EP-RT) by hybridizing 3′ end to RT template sequence
EP-RT
(d)
(c)
gRNA
5′
3′
3′
Cas9
(d) synthesis of mutagenized ssDNA by EP-RT
3′
5′
gRNA
3′
Cas9
(e) release napDNAbp
(g) drive process towards product formation (optional)
5′ endogenous flap removal
second strand nicking
Product
3′
3′ flap species
(f) Incorporate single mutagenized sequence into corresponding endogenous DNA
replication/repair
Mutagenized sequence incorporated into both strands of target locus

FIG. 22

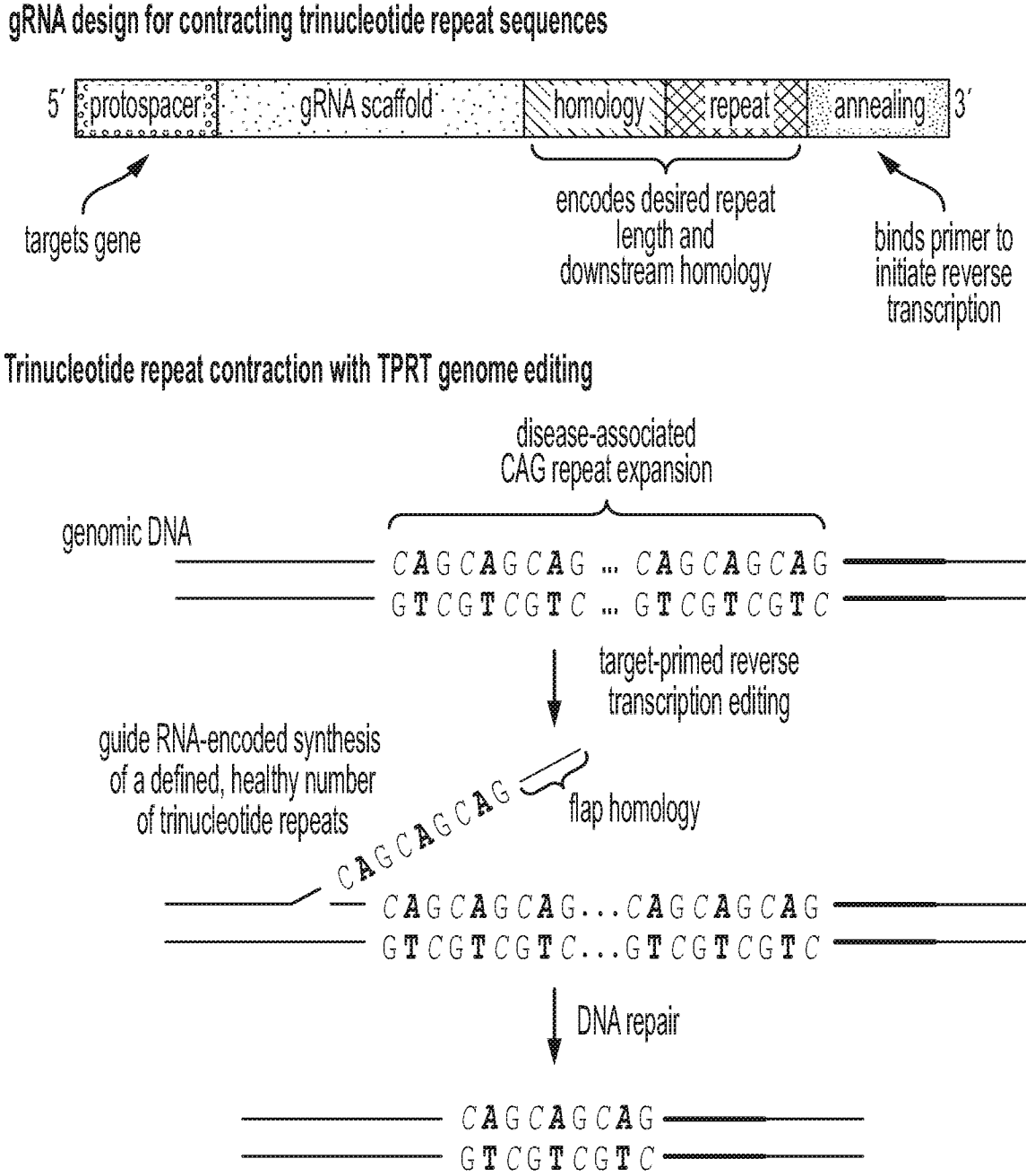

gRNA design for contracting trinucleotide repeat sequences
5′
protospacer
gRNA scaffold
homology
repeat
annealing
3′
targets gene
encodes desired repeat length and downstream homology
binds primer to initiate reverse transcription
Trinucleotide repeat contraction with TPRT genome editing
disease-associated CAG repeat expansion
genomic DNA
CAGCAGCAG ... CAGCAGCAG
GTCGTCGTC ... GTCGTCGTC
target-primed reverse transcription editing
guide RNA-encoded synthesis of a defined, healthy number of trinucleotide repeats
CAGCAGCAG
flap homology
CAGCAGCAG...CAGCAGCAG
GTCGTCGTC...GTCGTCGTC
DNA repair
CAGCAGCAG
GTCGTCGTC

FIG. 23

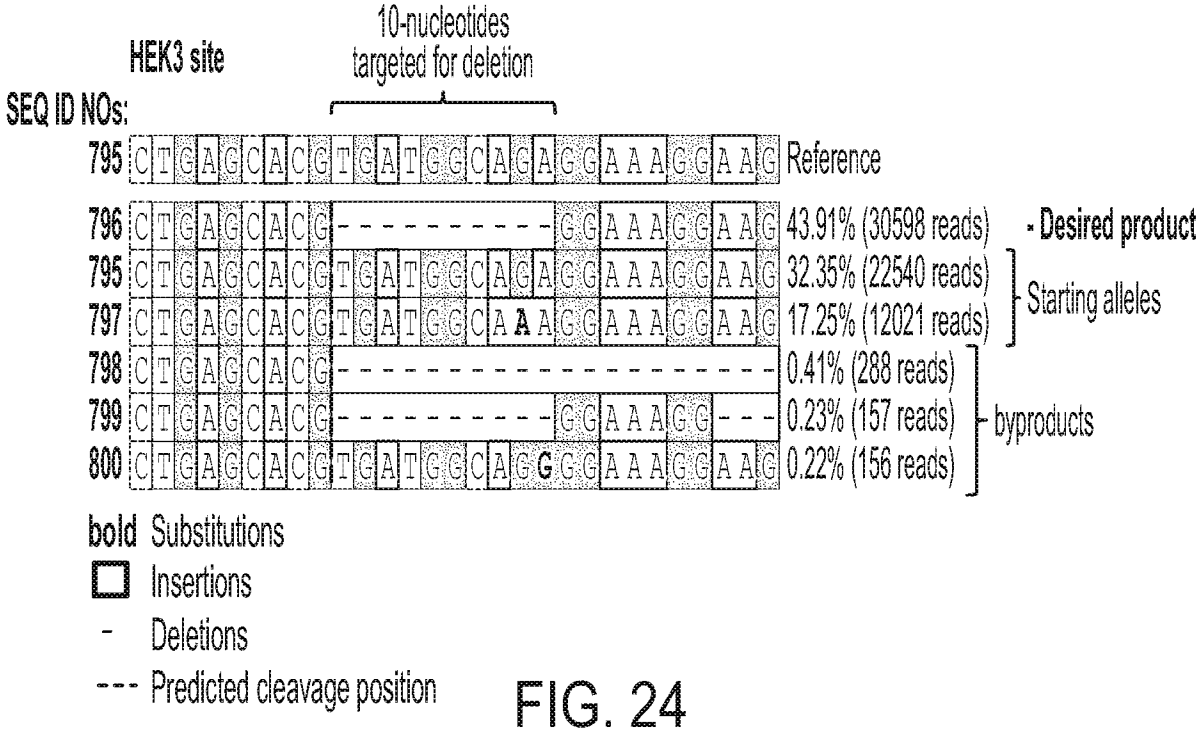

HEK3 site
10-nucleotides targeted for deletion
SEQ ID NOs:
795 CTGAGCACGTGATGGCAGAGGAAAGGAAG Reference
796 CTGAGCACG----------GGAAAGGAAG 43.91% (30598 reads) - Desired product
795 CTGAGCACGTGATGGCAGAGGAAAGGAAG 32.35% (22540 reads) Starting alleles
797 CTGAGCACGTGATGGCAAAAGGAAAGGAAG 17.25% (12021 reads) Starting alleles
798 CTGAGCACG---------- 0.41% (288 reads) byproducts
799 CTGAGCACG----------GGAAAGG--- 0.23% (157 reads) byproducts
800 CTGAGCACGTGATGGCAGGGGAAAGGAAG 0.22% (156 reads) byproducts
bold Substitutions
Insertions
- Deletions
--- Predicted cleavage position

FIG. 24

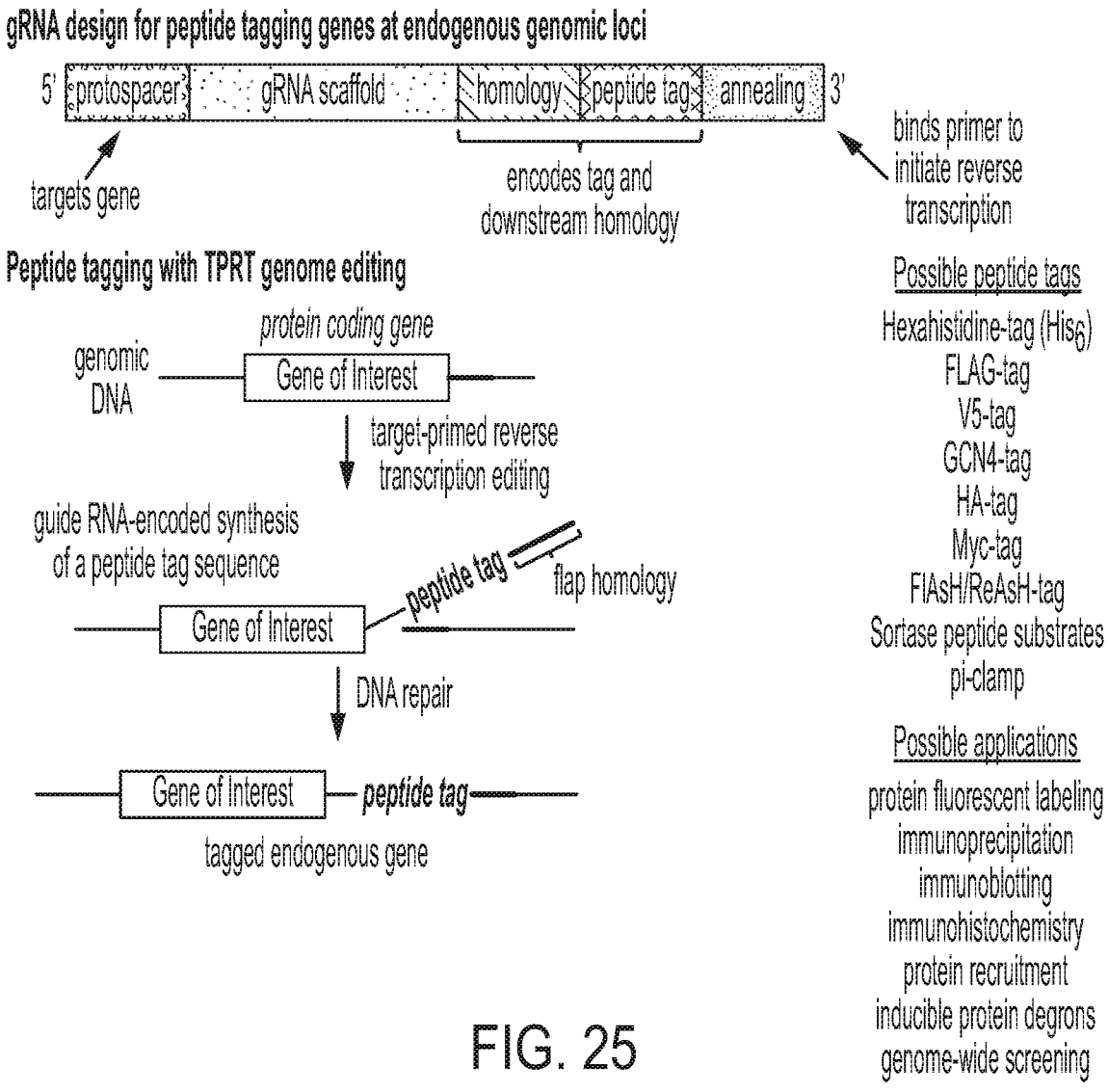

gRNA design for peptide tagging genes at endogenous genomic loci
5' protospacer gRNA scaffold homology peptide tag annealing 3'
targets gene
encodes tag and downstream homology
binds primer to initiate reverse transcription
Peptide tagging with TPRT genome editing
protein coding gene
genomic DNA
Gene of Interest
target-primed reverse transcription editing
guide RNA-encoded synthesis of a peptide tag sequence
peptide tag
flap homology
Gene of Interest
DNA repair
Gene of Interest peptide tag
tagged endogenous gene
Possible peptide tags
Hexahistidine-tag (His6)
FLAG-tag
V5-tag
GCN4-tag
HA-tag
Myc-tag
FlAsH/ReAsH-tag
Sortase peptide substrates
pi-clamp
Possible applications
protein fluorescent labeling
immunoprecipitation
immunoblotting
immunohistochemistry
protein recruitment
inducible protein degrons
genome-wide screening

FIG. 25

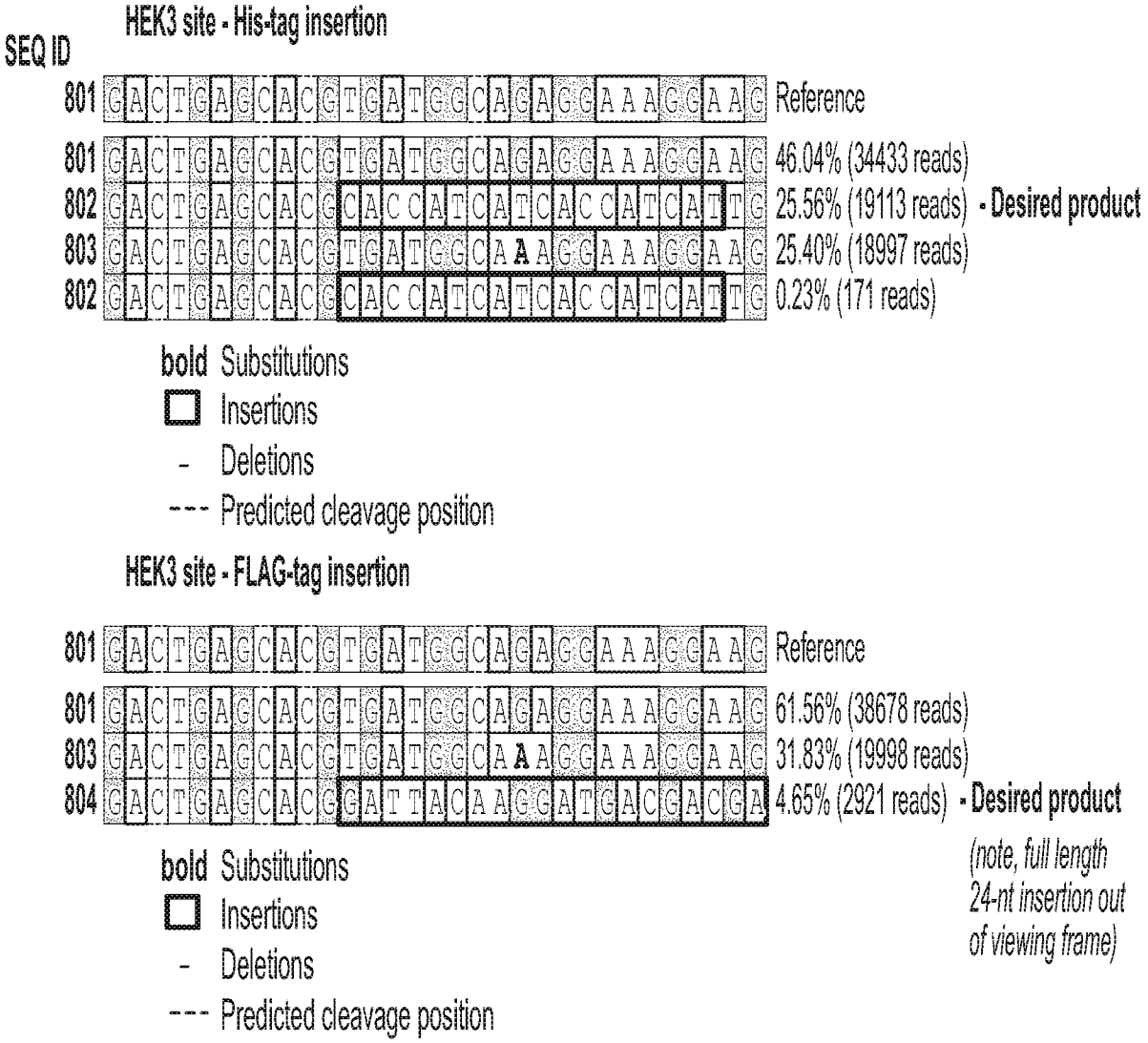


FIG. 26A

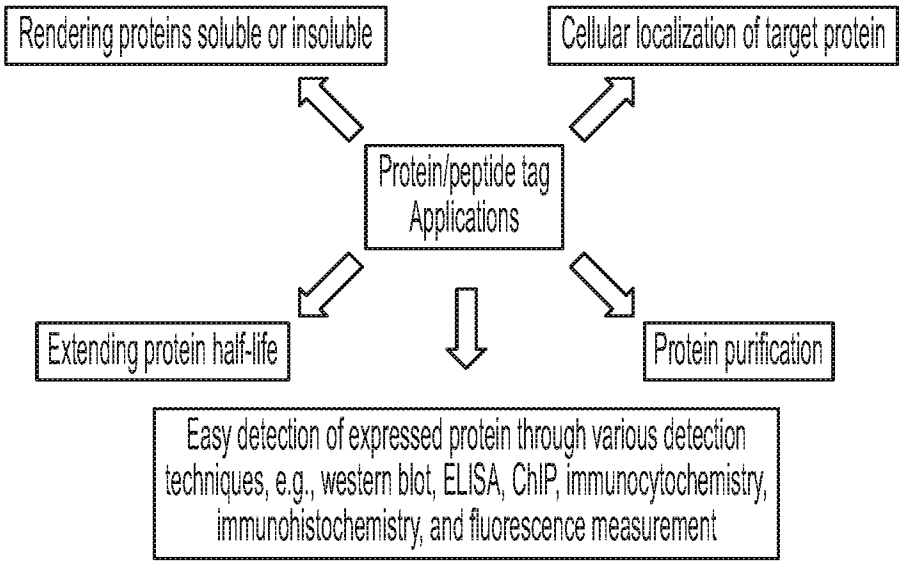


FIG. 26B

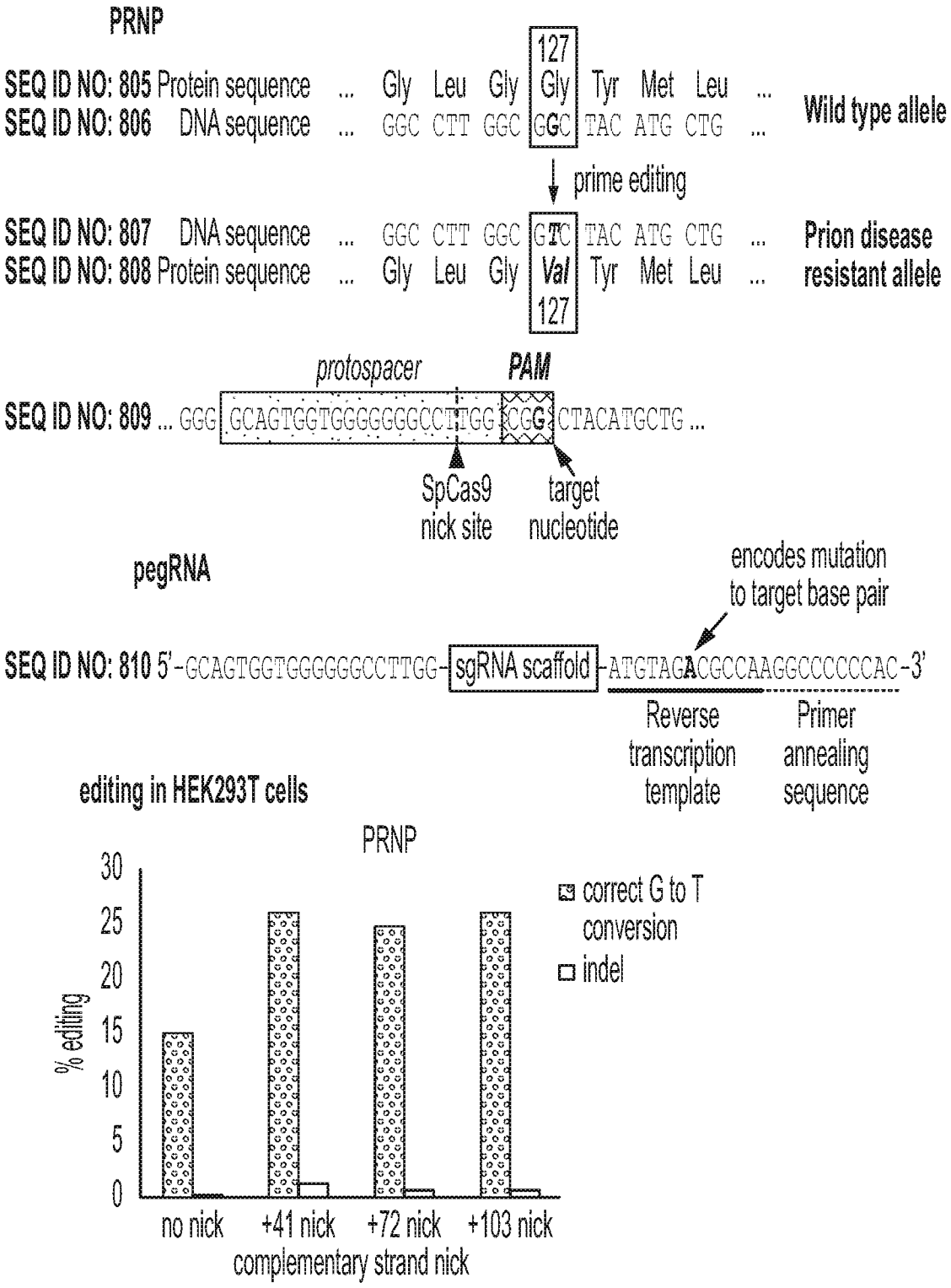

PRNP
SEQ ID NO: 805 Protein sequence ... Gly Leu Gly Gly Tyr Met Leu ...
SEQ ID NO: 806 DNA sequence ... GGC CTT GGC GGC TAC ATG CTG ...
127
Wild type allele
prime editing
SEQ ID NO: 807 DNA sequence ... GGC CTT GGC GTC TAC ATG CTG ...
SEQ ID NO: 808 Protein sequence ... Gly Leu Gly Val Tyr Met Leu ...
127
Prion disease resistant allele
protospacer
PAM
SEQ ID NO: 809 ... GGG GCAGTGGTGGGGGGGCCTTGG CGG CTACATGCTG ...
SpCas9 nick site
target nucleotide
pegRNA
encodes mutation to target base pair
SEQ ID NO: 810 5'-GCAGTGGTGGGGGGCCTTGG-sgRNA scaffold-ATGTAGACGCCAAGGCCCCCCAC-3'
Reverse transcription template
Primer annealing sequence
editing in HEK293T cells
PRNP
% editing
30
25
20
15
10
5
0
correct G to T conversion
indel
no nick
+41 nick
+72 nick
+103 nick
complementary strand nick

FIG. 27

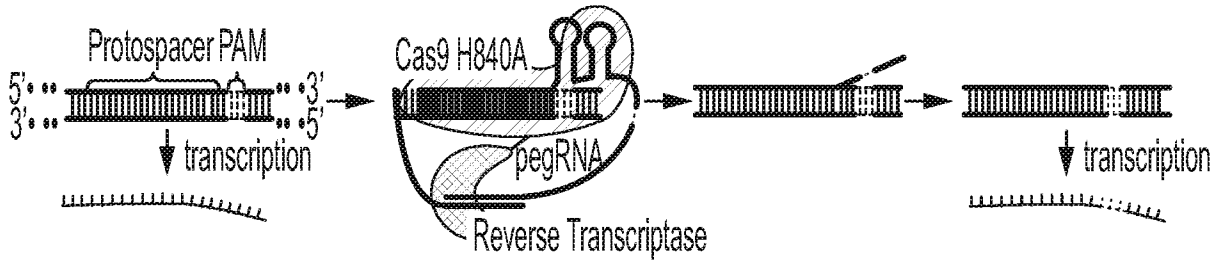


FIG. 28A

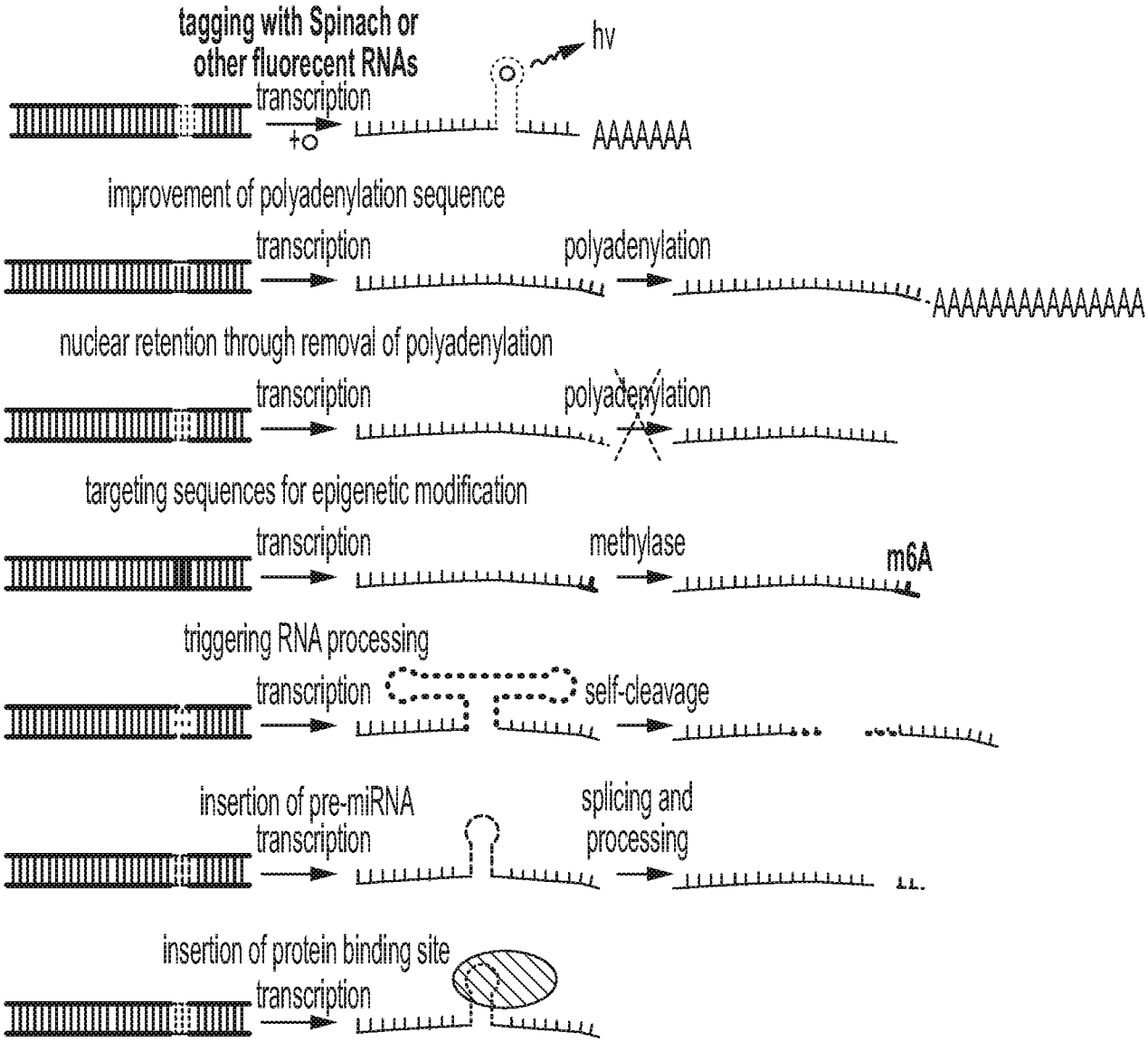


FIG. 28B

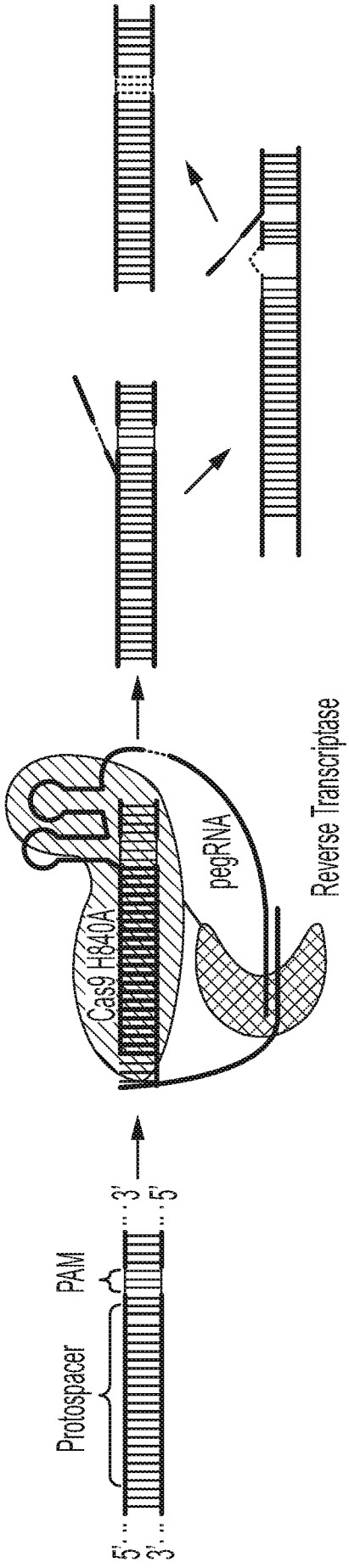

Protospacer
PAM
3'
5'
5'
3'
Cas9 H840A
pegRNA
Reverse Transcriptase

FIG. 29A

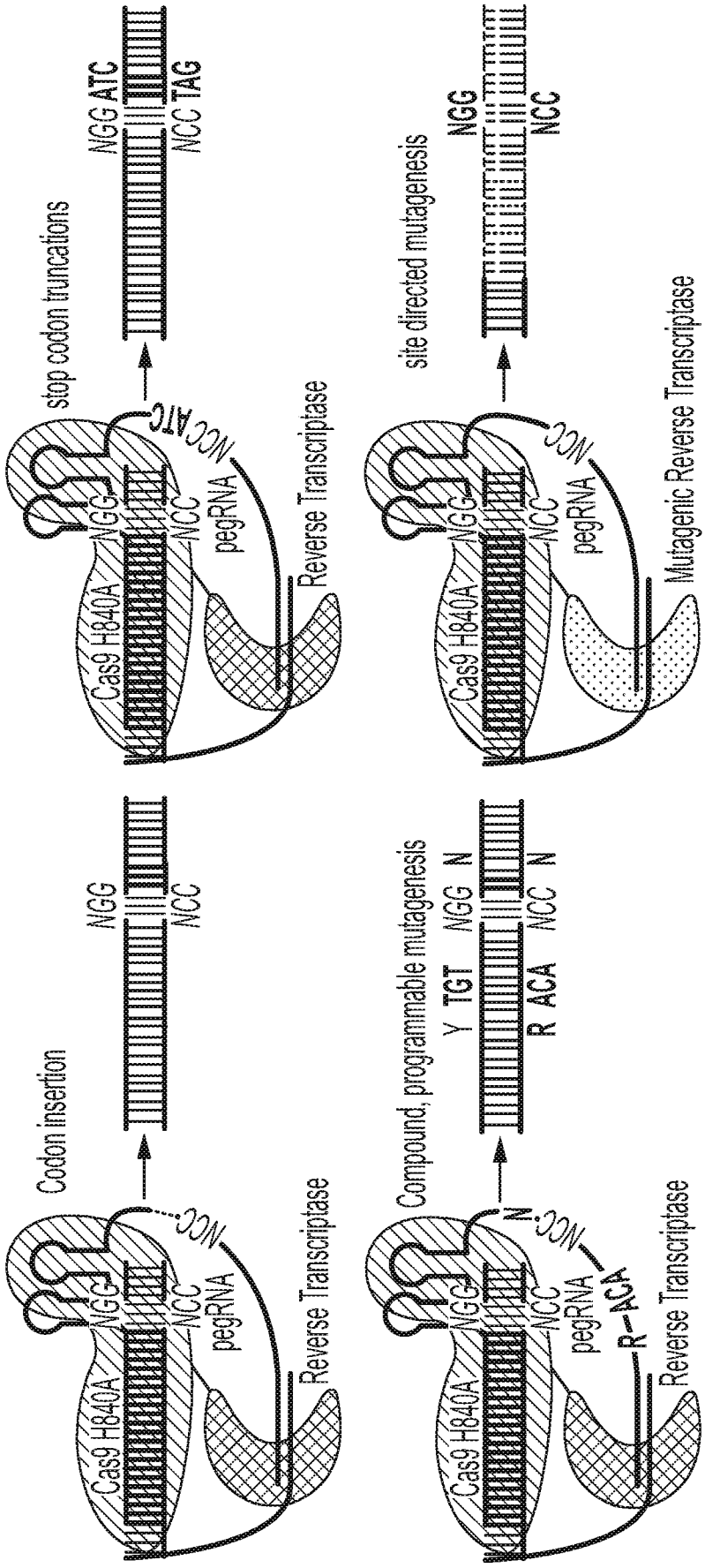

stop codon truncations
NGG ATC
NCC TAG
Cas9 H840A
NGG
NCC
pegRNA
NCC ATC
Reverse Transcriptase
site directed mutagenesis
NGG
NCC
Mutagenic Reverse Transcriptase
Codon insertion
NGG
NCC
Reverse Transcriptase
Compound, programmable mutagenesis
Y TGT NGG N
R ACA NCC N
N
R—ACA

FIG. 29B

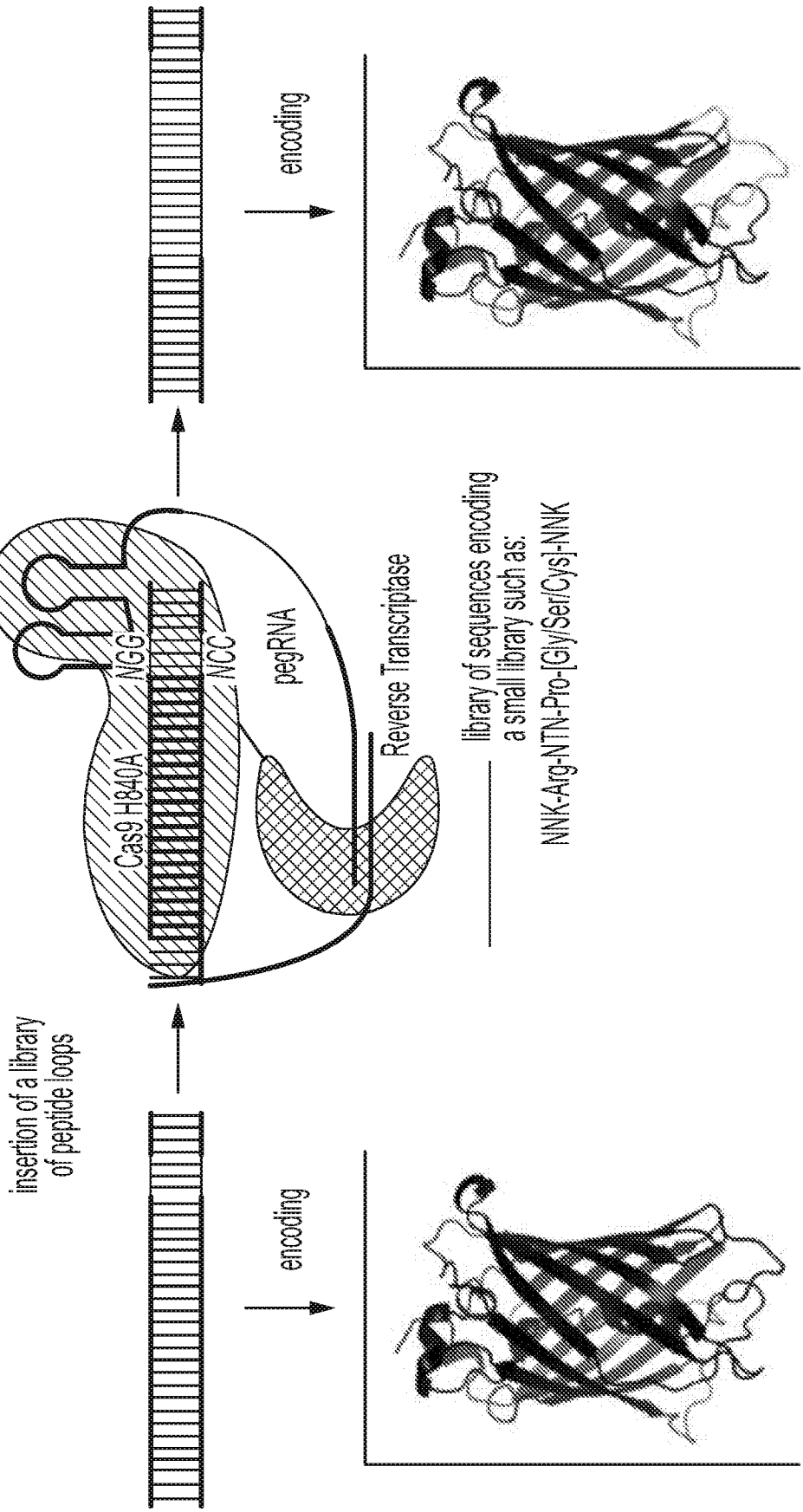

insertion of a library of peptide loops
encoding
encoding
Cas9 H840A
NGG
NCC
pegRNA
Reverse Transcriptase
library of sequences encoding a small library such as:
NNK-Arg-NTN-Pro-[Gly/Ser/Cys]-NNK

FIG. 29C

programmable codon deletion and truncations

SEQ ID NO:811	GFP	NFKIRHNIEDGSVQLADHYQQNTPI
SEQ ID NO:812	del5	NFKI-HNIEDGSVQLADHYQQNTPI
SEQ ID NO:813	del6	NFKIR-NIEDGSVQLADHYQQNTPI
SEQ ID NO:814	del8	NFKIRHN-EDGSVQLADHYQQNTPI
SEQ ID NO:815	del 8-13	NFKIRHN------QLADHYQQNTPI
SEQ ID NO:816	del 1-5	-----HNIEDGSVQLADHYQQNTPI
SEQ ID NO:817	del 15-25	NFKIRHNIEDGSVQ----------

FIG. 29D

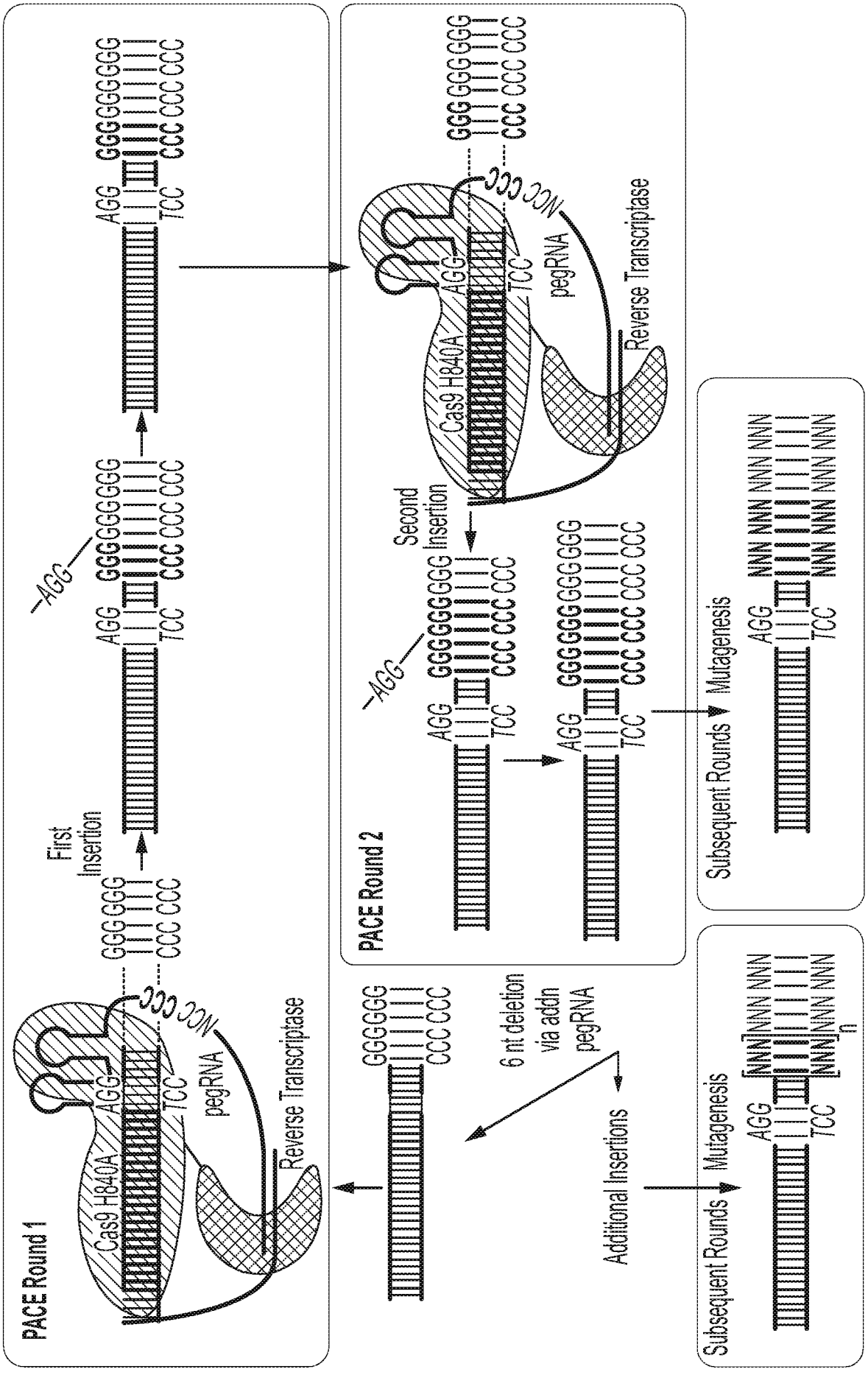

PACE Round 1
Cas9 H840A
pegRNA
Reverse Transcriptase
First Insertion
PACE Round 2
Second Insertion
6 nt deletion via addn pegRNA
Additional Insertions
Subsequent Rounds
Mutagenesis
Subsequent Rounds
Mutagenesis

FIG. 30

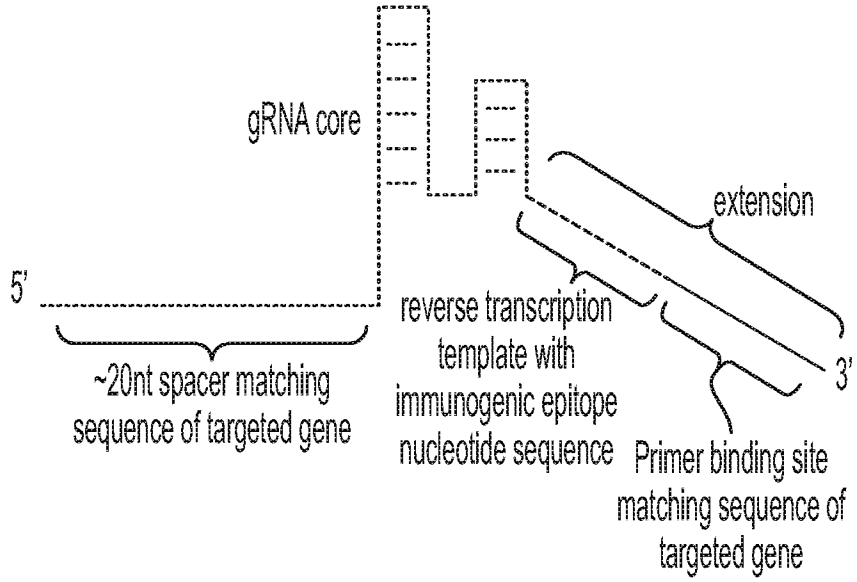

gRNA core
extension
5'
reverse transcription template with immunogenic epitope nucleotide sequence
3'
~20nt spacer matching sequence of targeted gene
Primer binding site matching sequence of targeted gene

FIG. 31

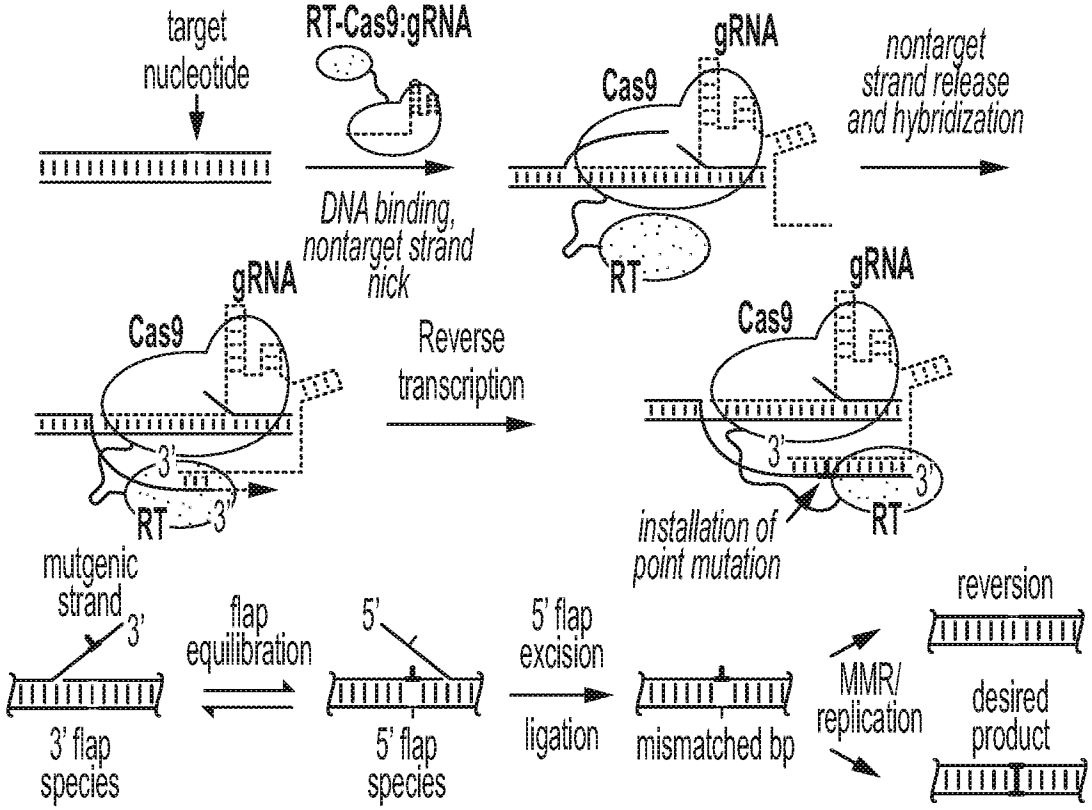

target nucleotide
RT-Cas9:gRNA
DNA binding, nontarget strand nick
gRNA
Cas9
RT
nontarget strand release and hybridization
Reverse transcription
3'
3''
installation of point mutation
mutgenic strand
flap equilibration
5'
5' flap excision
ligation
reversion
MMR/ replication
desired product
mismatched bp
3' flap species
5' flap species

FIG. 32

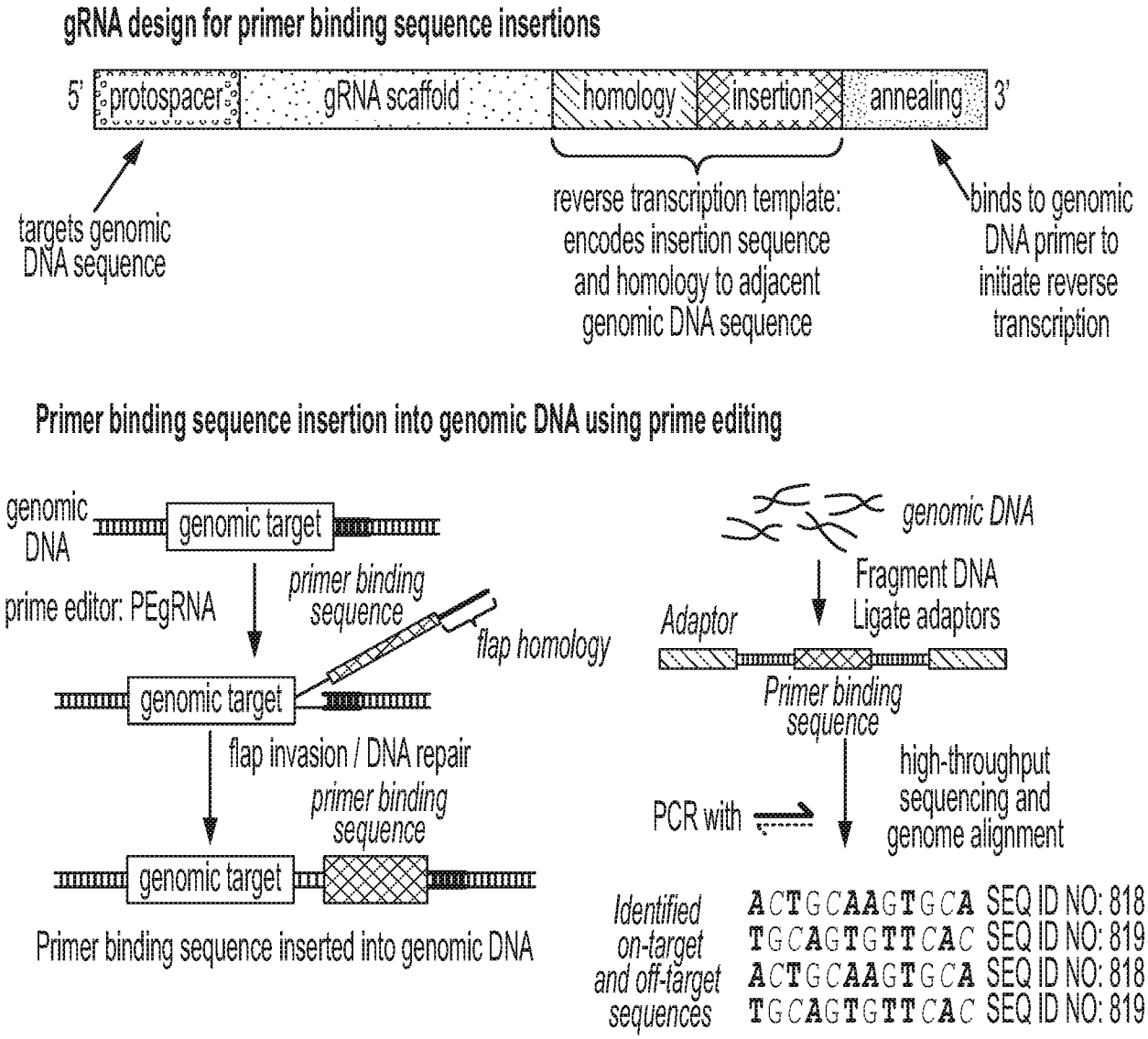

gRNA design for primer binding sequence insertions
5'
protospacer
gRNA scaffold
homology
insertion
annealing
3'
targets genomic DNA sequence
reverse transcription template: encodes insertion sequence and homology to adjacent genomic DNA sequence
binds to genomic DNA primer to initiate reverse transcription
Primer binding sequence insertion into genomic DNA using prime editing
genomic DNA
genomic target
prime editor: PEgRNA
primer binding sequence
flap homology
genomic target
flap invasion / DNA repair
primer binding sequence
genomic target
Primer binding sequence inserted into genomic DNA
genomic DNA
Fragment DNA
Ligate adaptors
Adaptor
Primer binding sequence
PCR with
high-throughput sequencing and genome alignment
Identified on-target and off-target sequences
ACTGCAAGTGCA SEQ ID NO: 818
TGCAGTGTTCAC SEQ ID NO: 819
ACTGCAAGTGCA SEQ ID NO: 818
TGCAGTGTTCAC SEQ ID NO: 819

FIG. 33

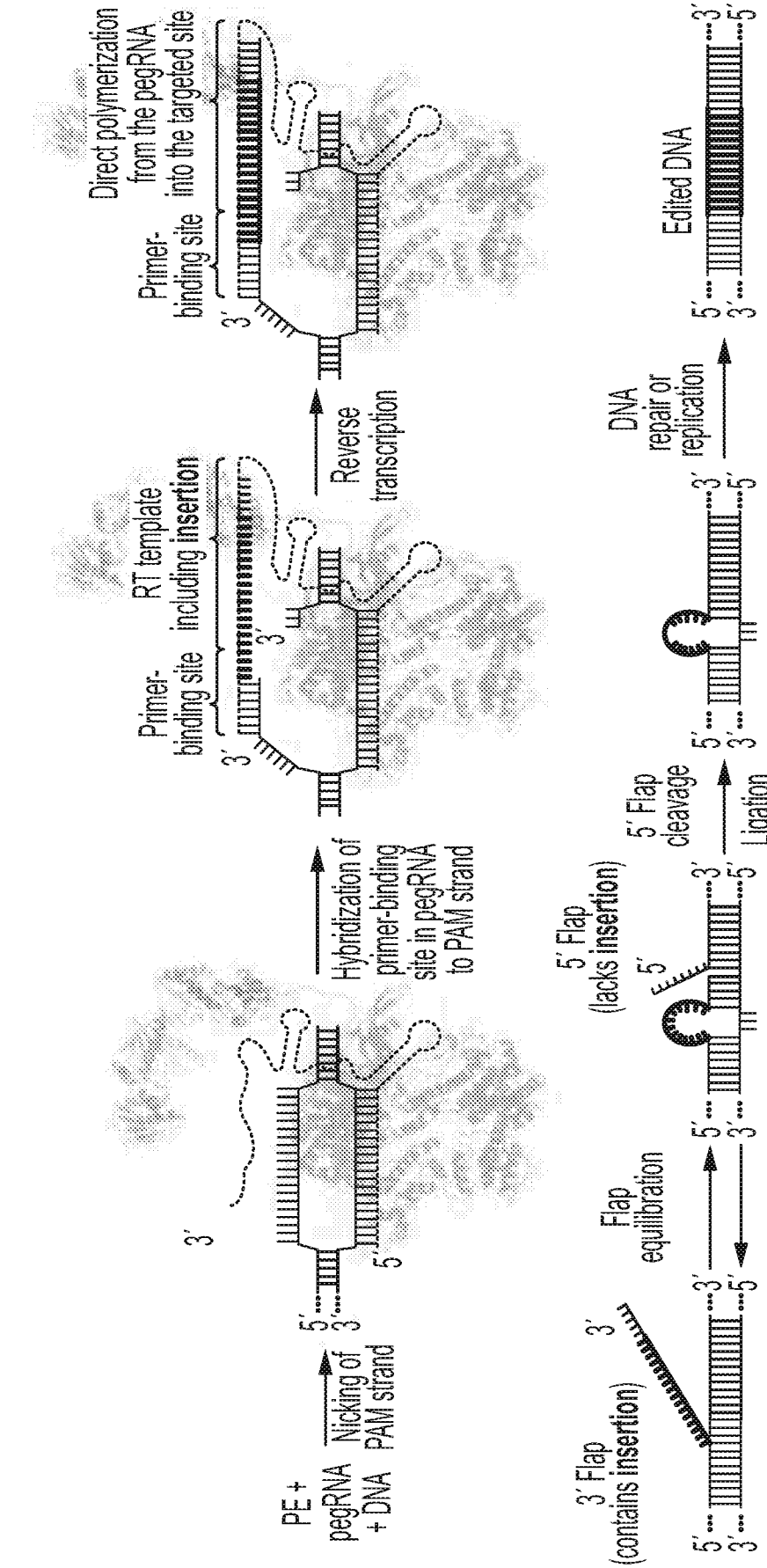

PE + pegRNA + DNA
Nicking of PAM strand
Hybridization of primer-binding site in pegRNA to PAM strand
Primer-binding site
RT template including insertion
Reverse transcription
Primer-binding site
Direct polymerization from the pegRNA into the targeted site
3′ Flap (contains insertion)
Flap equilibration
5′ Flap (lacks insertion)
5′ Flap cleavage
Ligation
DNA repair or replication
Edited DNA

FIG. 34

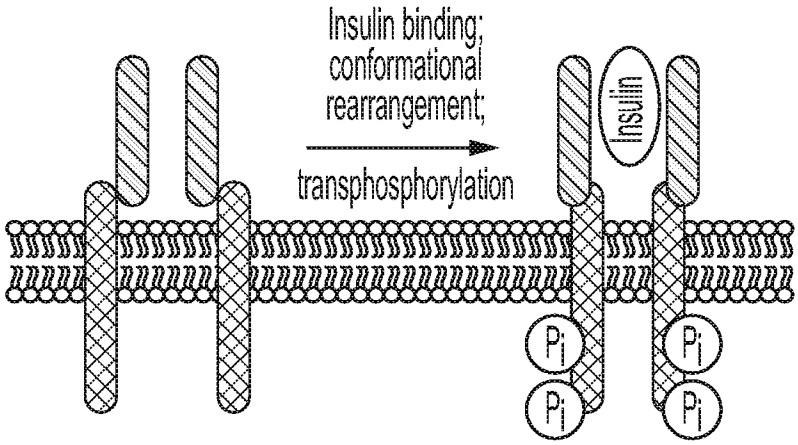


FIG. 35A

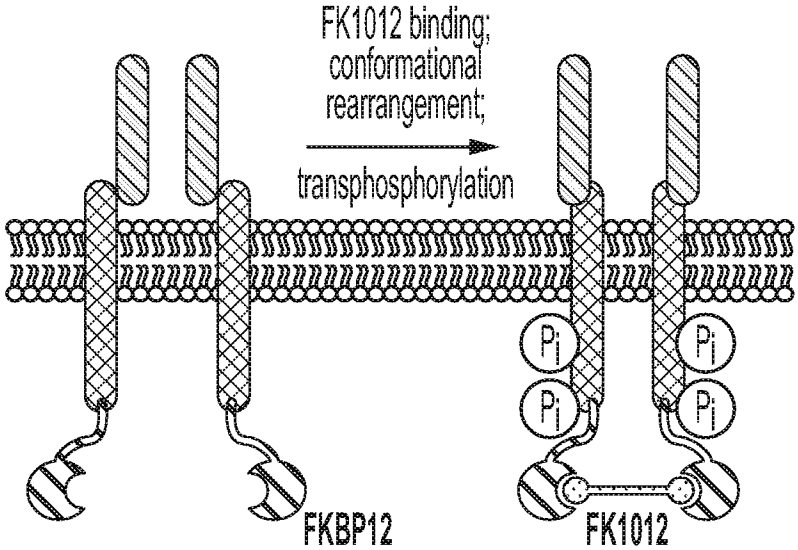
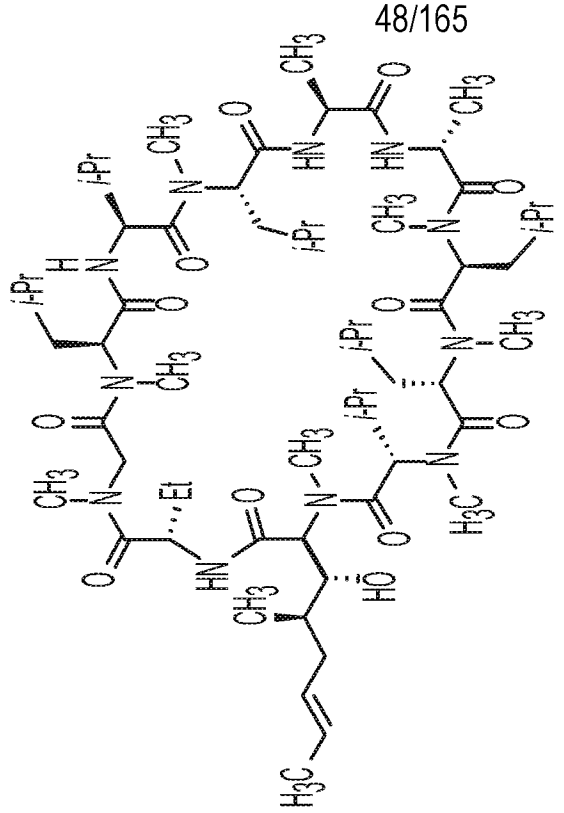
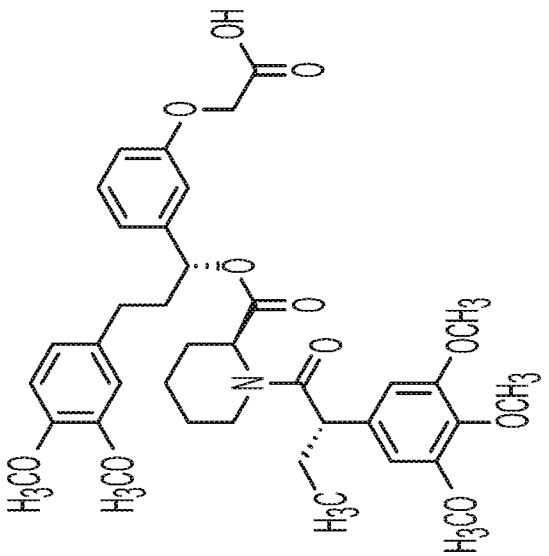


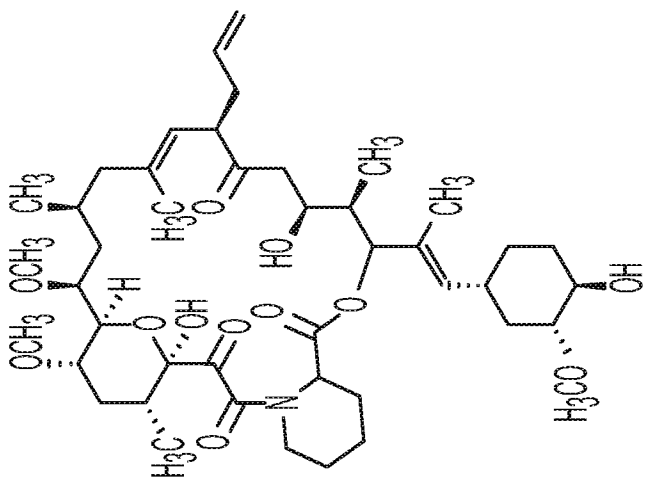
FIG. 35B



3
cyclosporin A
target: cyclophilin



2
bumped FK506 mimic
target: FKBP12 F36V



1
FK506
target: FKBP12

FIG. 36

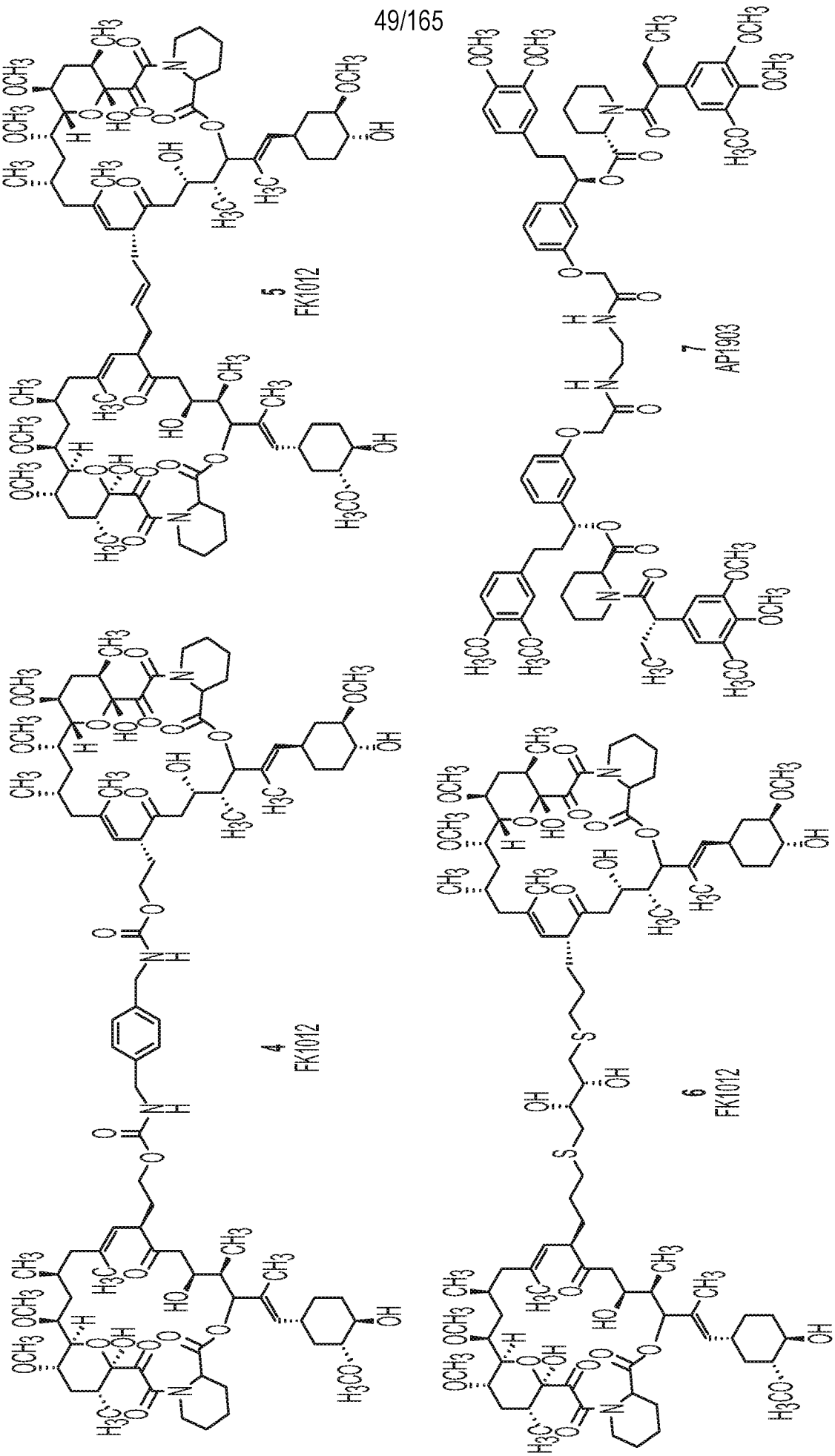
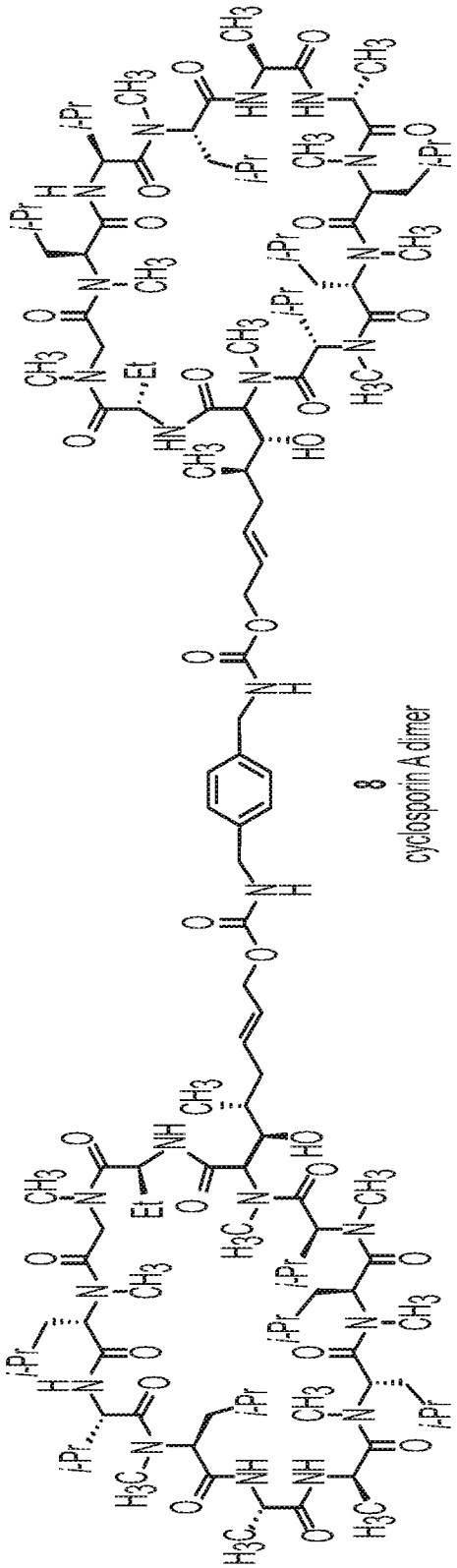
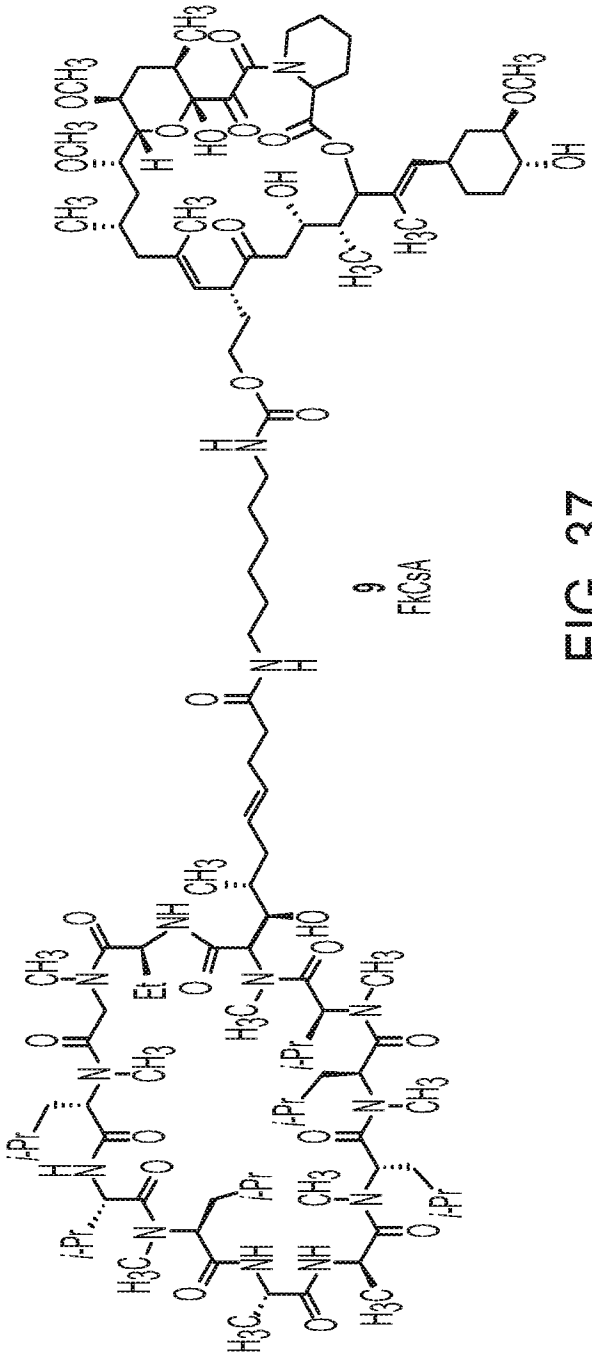


FIG. 37



8
cyclosporin A dimer



9
FKCsA

FIG. 37
CONTINUED

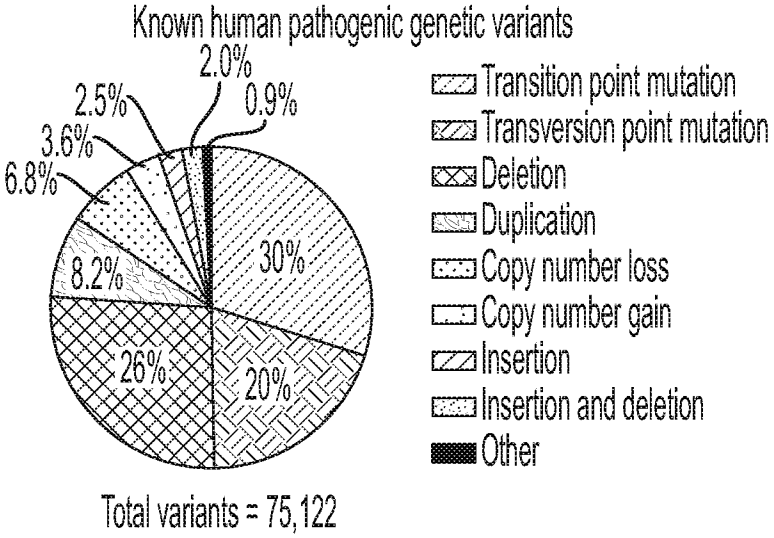


FIG. 38A

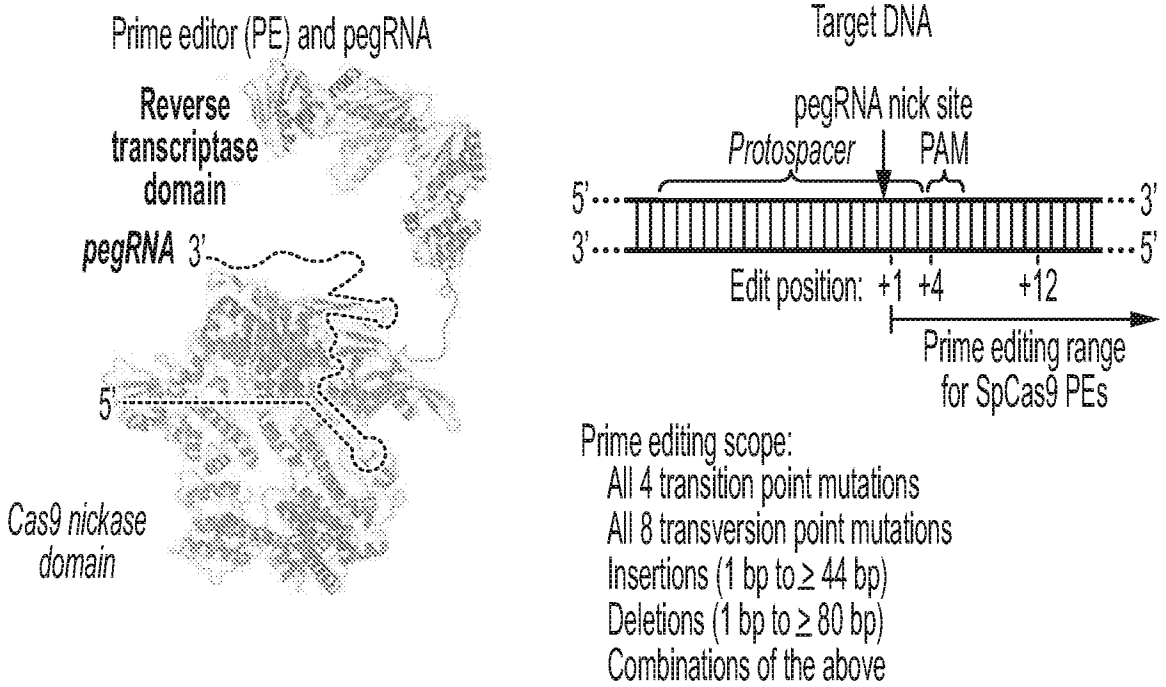


FIG. 38B

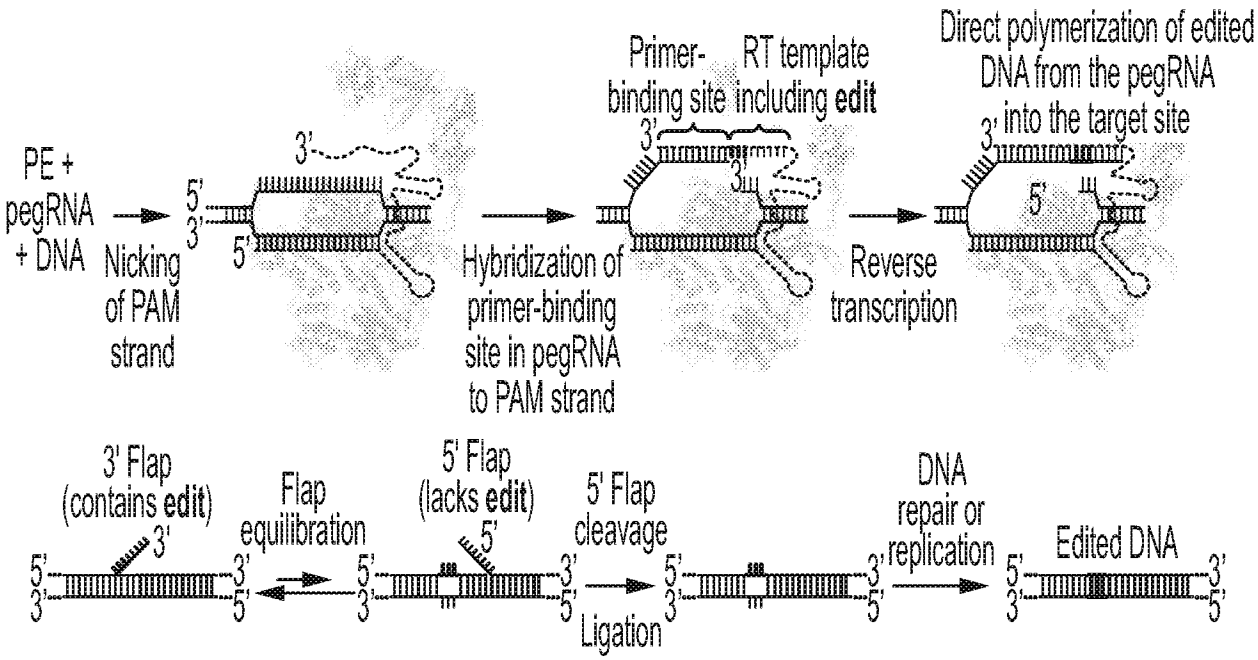


FIG. 38C

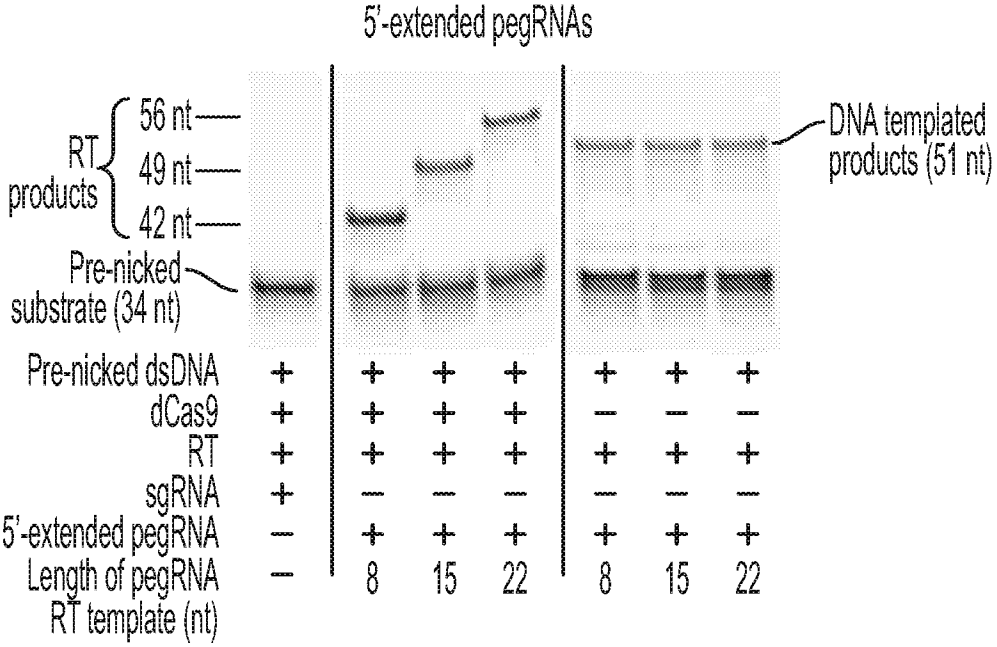


FIG. 38D

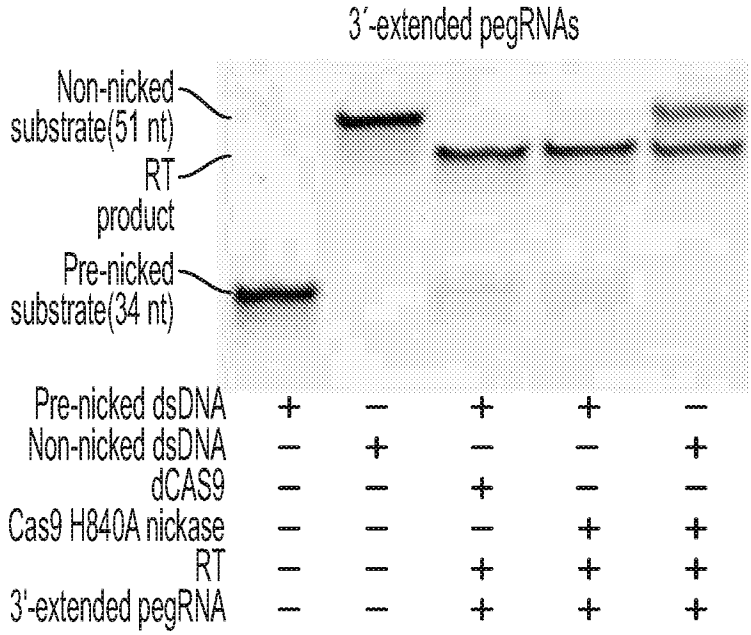


FIG. 38E

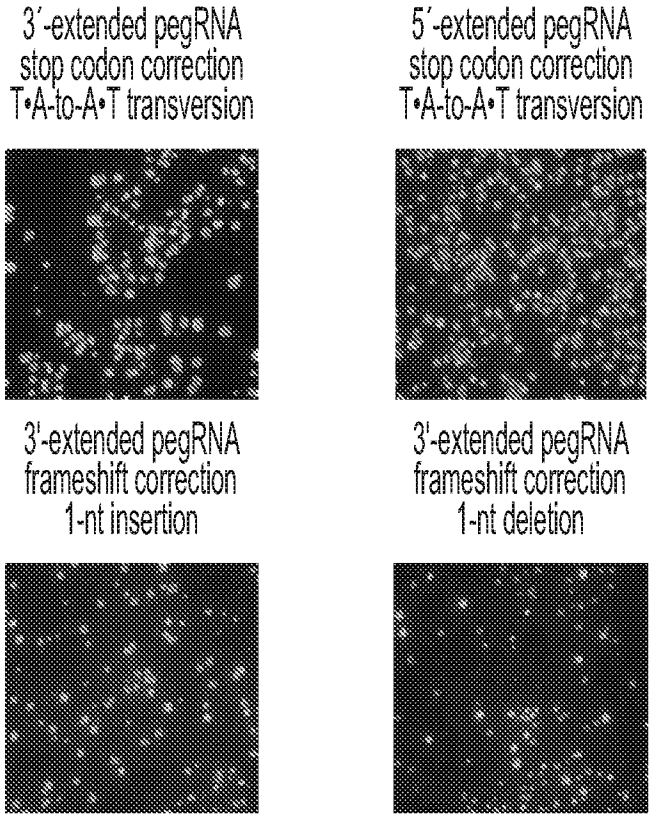


FIG. 38F

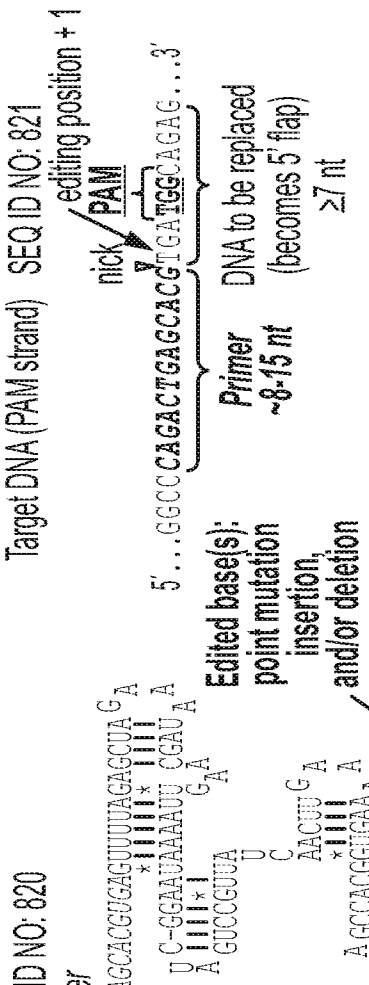


FIG. 39A

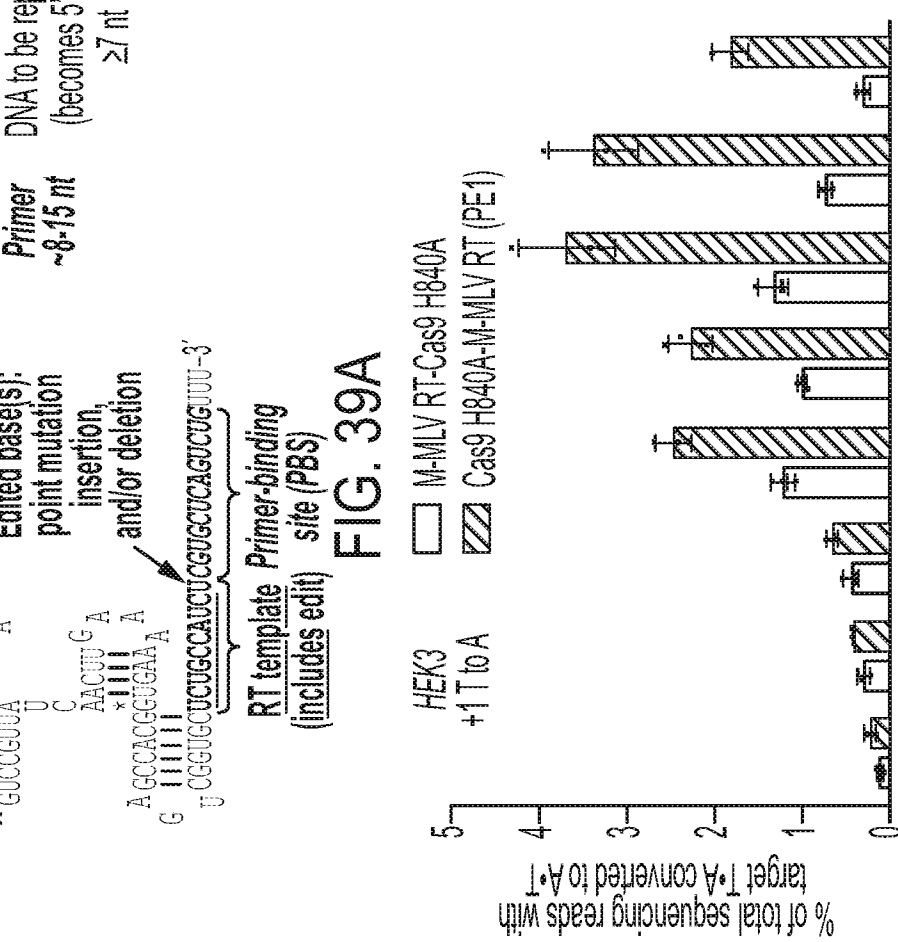


FIG. 39B

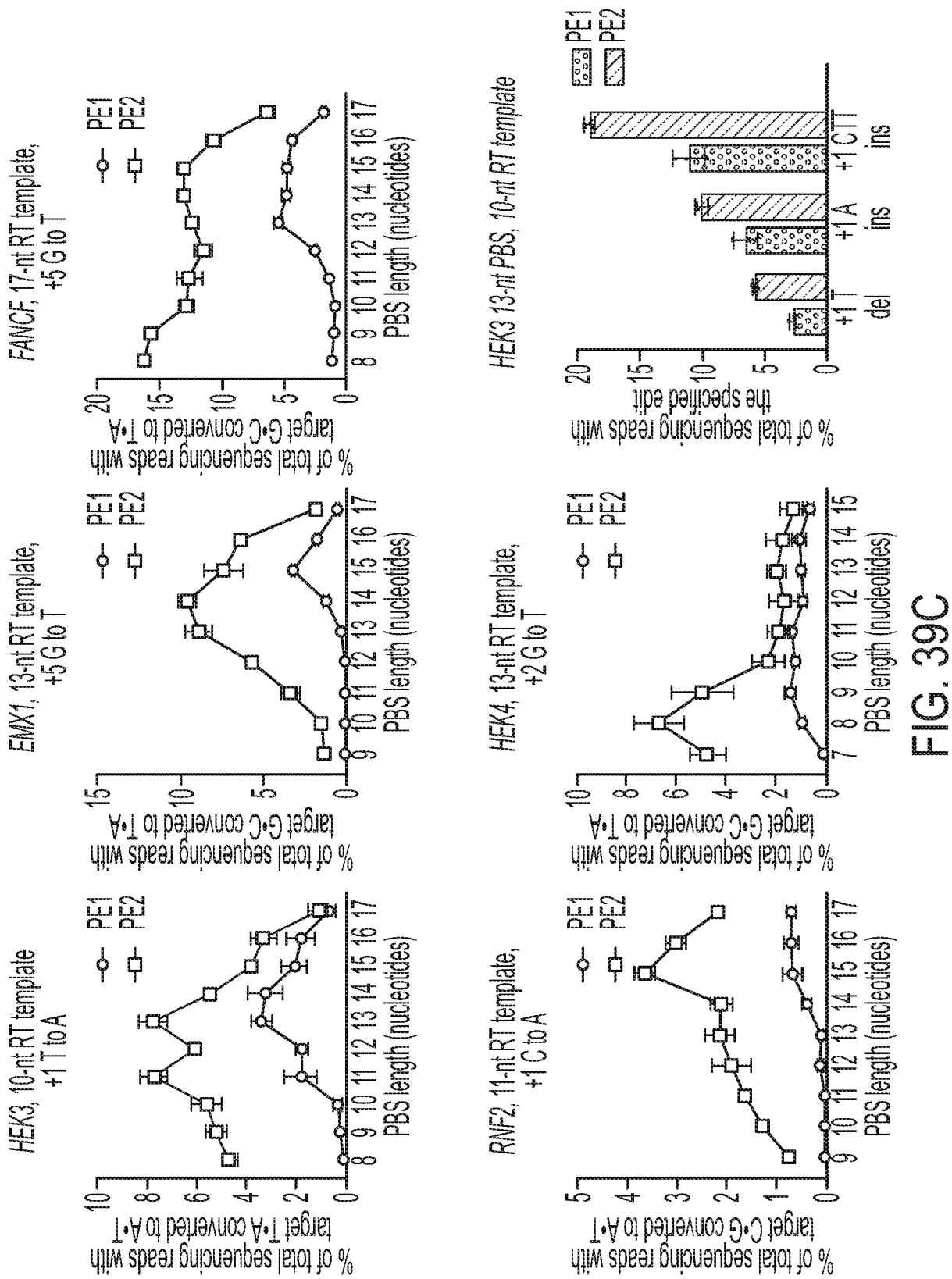

HEK3, 10-nt RT template, +1 T to A
EMX1, 13-nt RT template, +5 G to T
FANCF, 17-nt RT template, +5 G to T
RNF2, 11-nt RT template, +1 C to A
HEK4, 13-nt RT template, +2 G to T
HEK3 13-nt PBS, 10-nt RT template
PE1
PE2
% of total sequencing reads with target T•A converted to A•T
% of total sequencing reads with target G•C converted to T•A
% of total sequencing reads with target C•G converted to A•T
% of total sequencing reads with the specified edit
PBS length (nucleotides)
+1 T
del
+1 A
ins
+1 CTT
ins

FIG. 39C

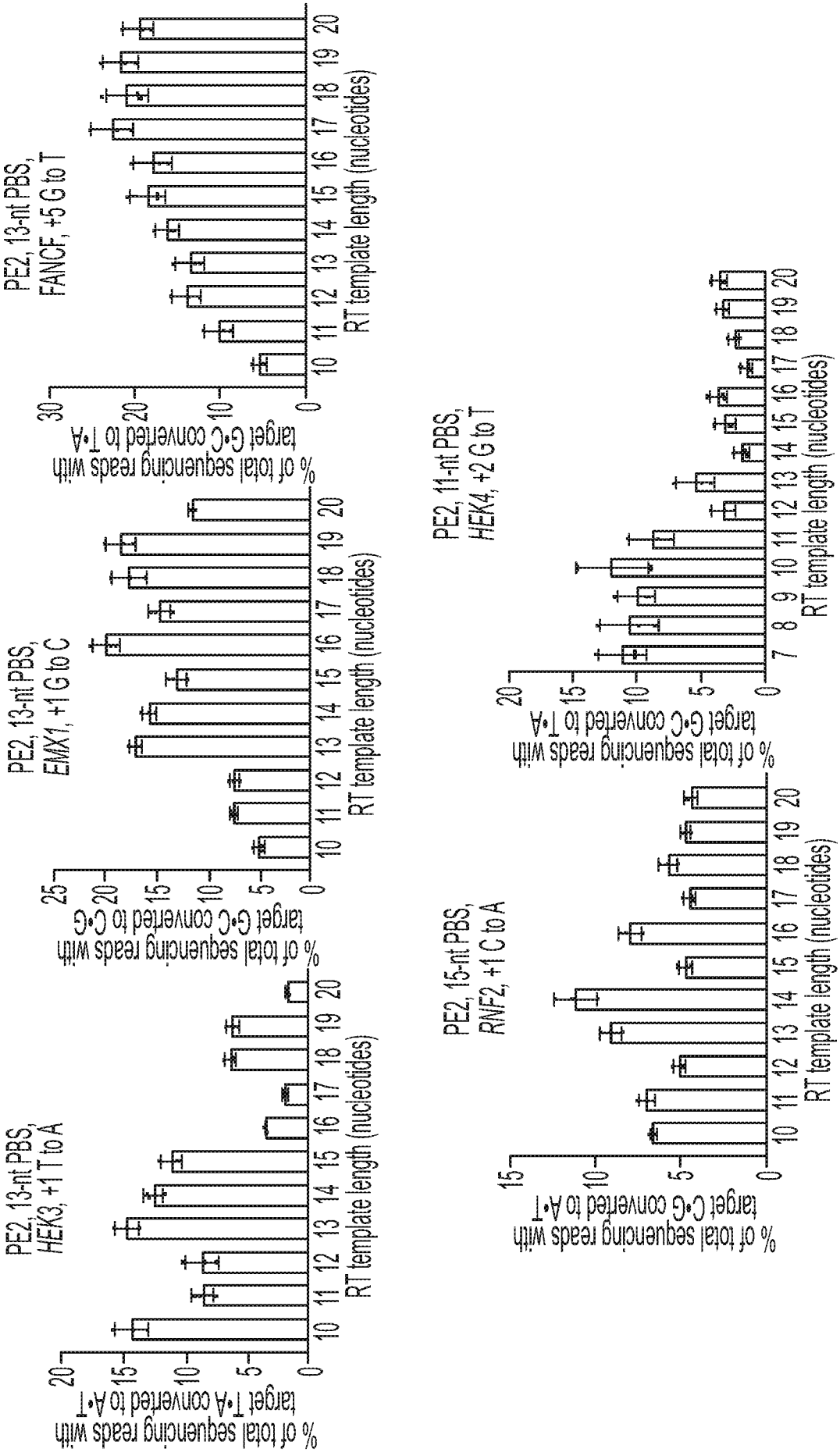

PE2, 13-nt PBS,
HEK3, +1 T to A
% of total sequencing reads with target T•A converted to A•T
RT template length (nucleotides)
PE2, 13-nt PBS,
EMX1, +1 G to C
% of total sequencing reads with target G•C converted to C•G
RT template length (nucleotides)
PE2, 13-nt PBS,
FANCF, +5 G to T
% of total sequencing reads with target G•C converted to T•A
RT template length (nucleotides)
PE2, 15-nt PBS,
RNF2, +1 C to A
% of total sequencing reads with target C•G converted to A•T
RT template length (nucleotides)
PE2, 11-nt PBS,
HEK4, +2 G to T
% of total sequencing reads with target G•C converted to T•A
RT template length (nucleotides)

FIG. 39D

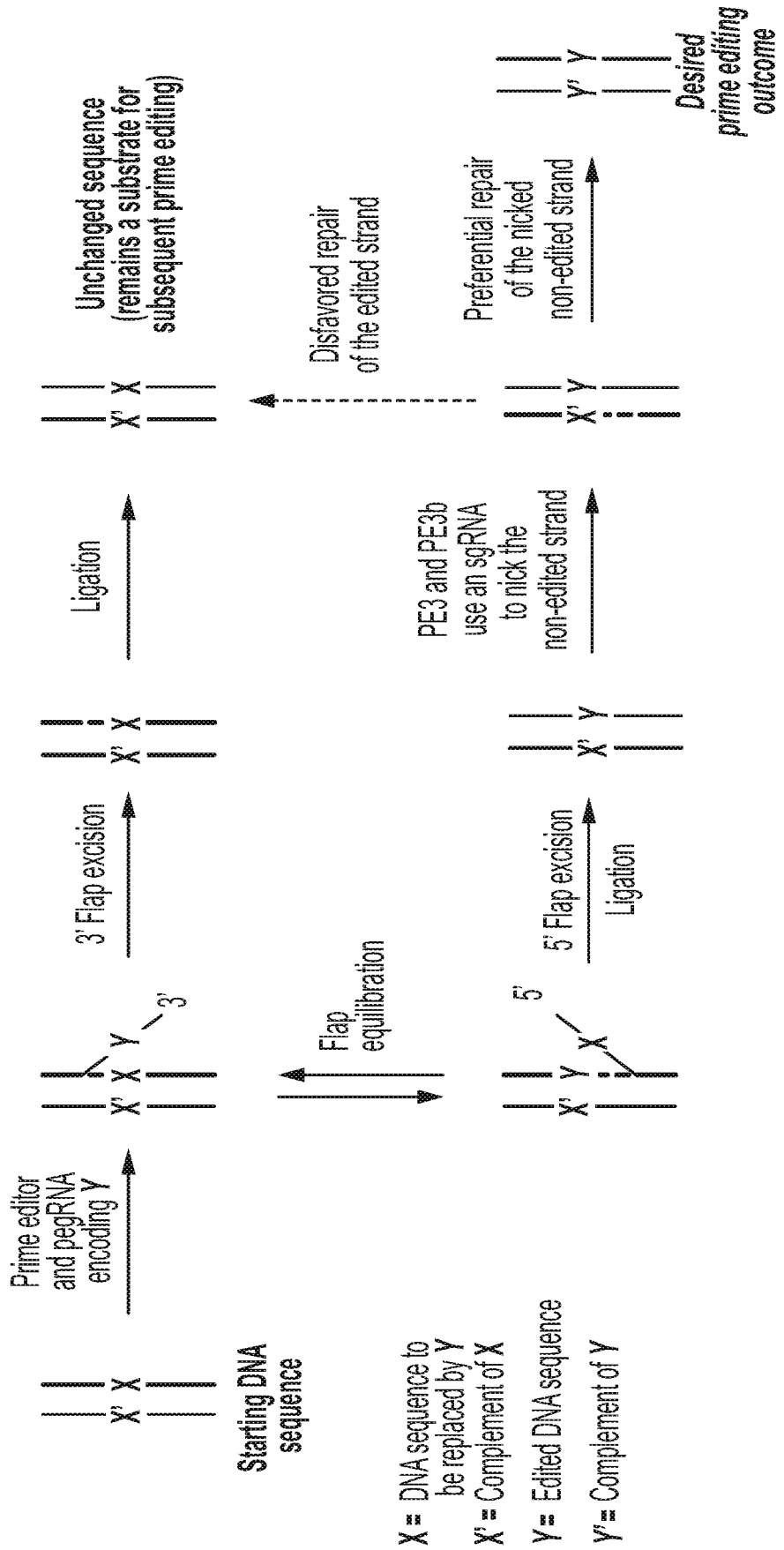

Prime editor and pegRNA encoding Y
Starting DNA sequence
3' Flap excision
Flap equilibration
5' Flap excision
Ligation
PE3 and PE3b use an sgRNA to nick the non-edited strand
Ligation
Disfavored repair of the edited strand
Preferential repair of the nicked non-edited strand
Unchanged sequence (remains a substrate for subsequent prime editing)
Desired prime editing outcome
X = DNA sequence to be replaced by Y
X' = Complement of X
Y = Edited DNA sequence
Y' = Complement of Y

FIG. 40A

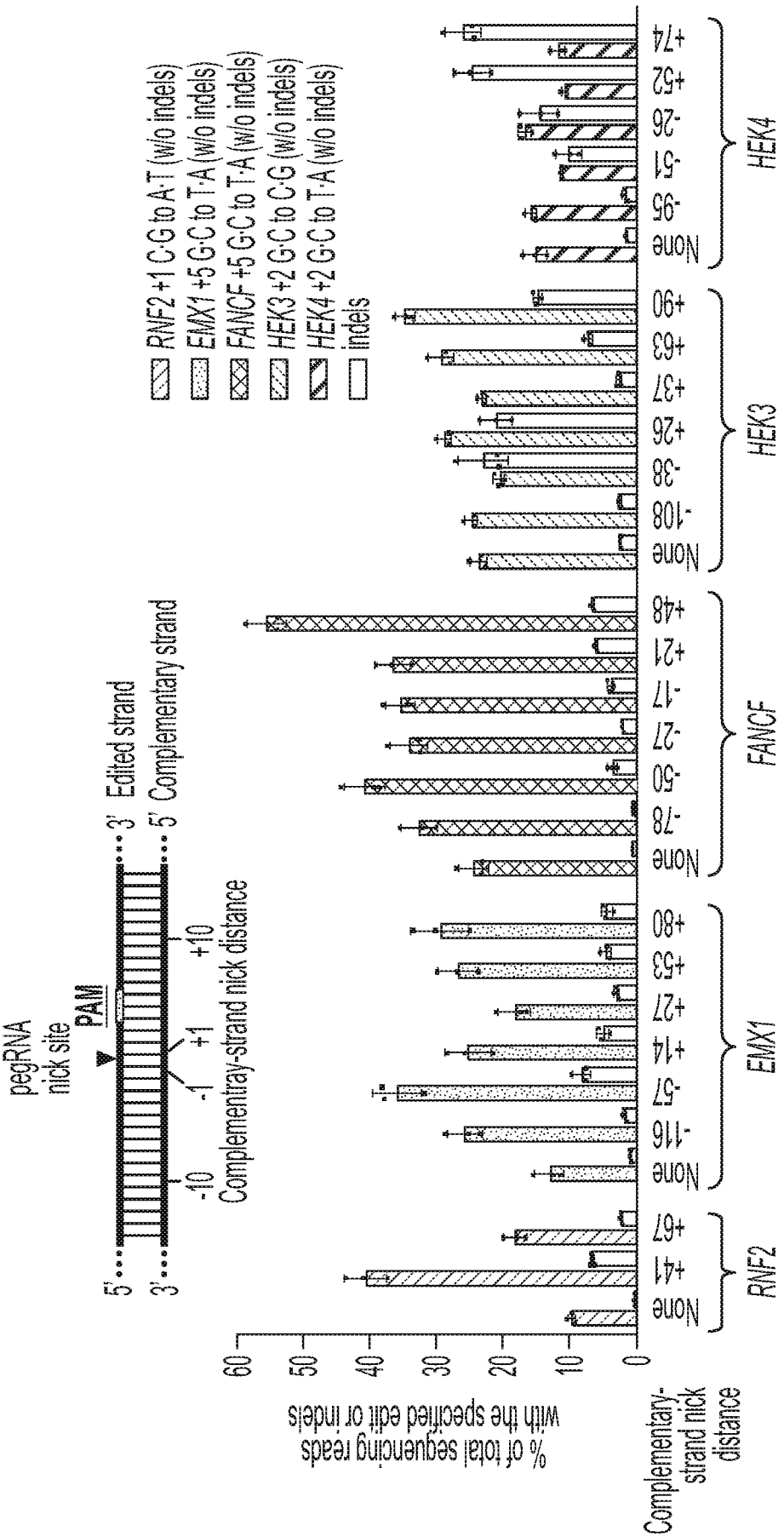

RNF2 +1 C·G to A·T (w/o indels)
EMX1 +5 G·C to T·A (w/o indels)
FANCF +5 G·C to T·A (w/o indels)
HEK3 +2 G·C to C·G (w/o indels)
HEK4 +2 G·C to T·A (w/o indels)
indels
pegRNA nick site
PAM
3' Edited strand
5' Complementary strand
Complementray-strand nick distance
% of total sequencing reads with the specified edit or indels
Complementary-strand nick distance
None +41 +67 RNF2
None -116 -57 +14 +27 +53 +80 EMX1
None -78 -50 -27 -17 +21 +48 FANCF
None -108 -38 +26 +37 +63 +90 HEK3
None -95 -51 -26 +52 +74 HEK4

FIG. 40B

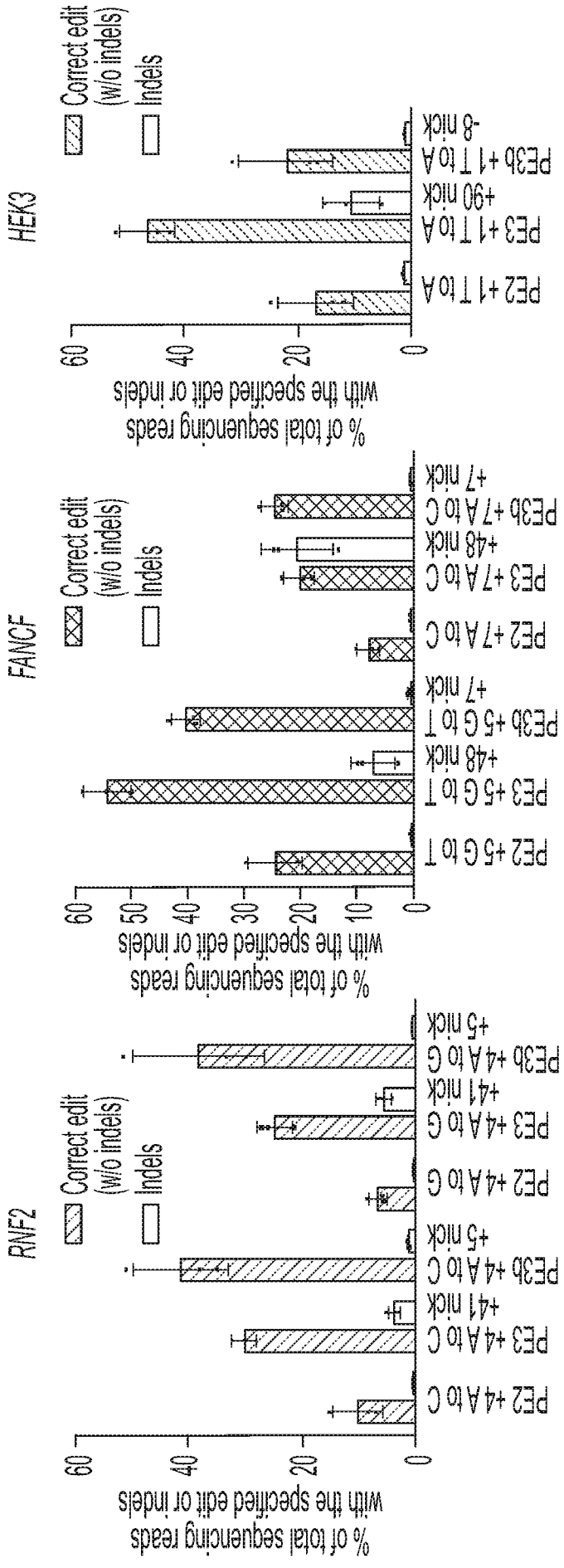

RNF2
FANCF
HEK3
Correct edit (w/o indels)
Indels
% of total sequencing reads with the specified edit or indels
PE2 +4 A to C
PE3 +4 A to C
+41 nick
PE3b +4 A to C
+5 nick
PE2 +4 A to G
PE3 +4 A to G
+41 nick
PE3b +4 A to G
+5 nick
PE2 +5 G to T
PE3 +5 G to T
+48 nick
PE3b +5 G to T
+7 nick
PE2 +7 A to C
PE3 +7 A to C
+48 nick
PE3b +7 A to C
+7 nick
PE2 +1 T to A
PE3 +1 T to A
+90 nick
PE3b +1 T to A
-8 nick

FIG. 40C

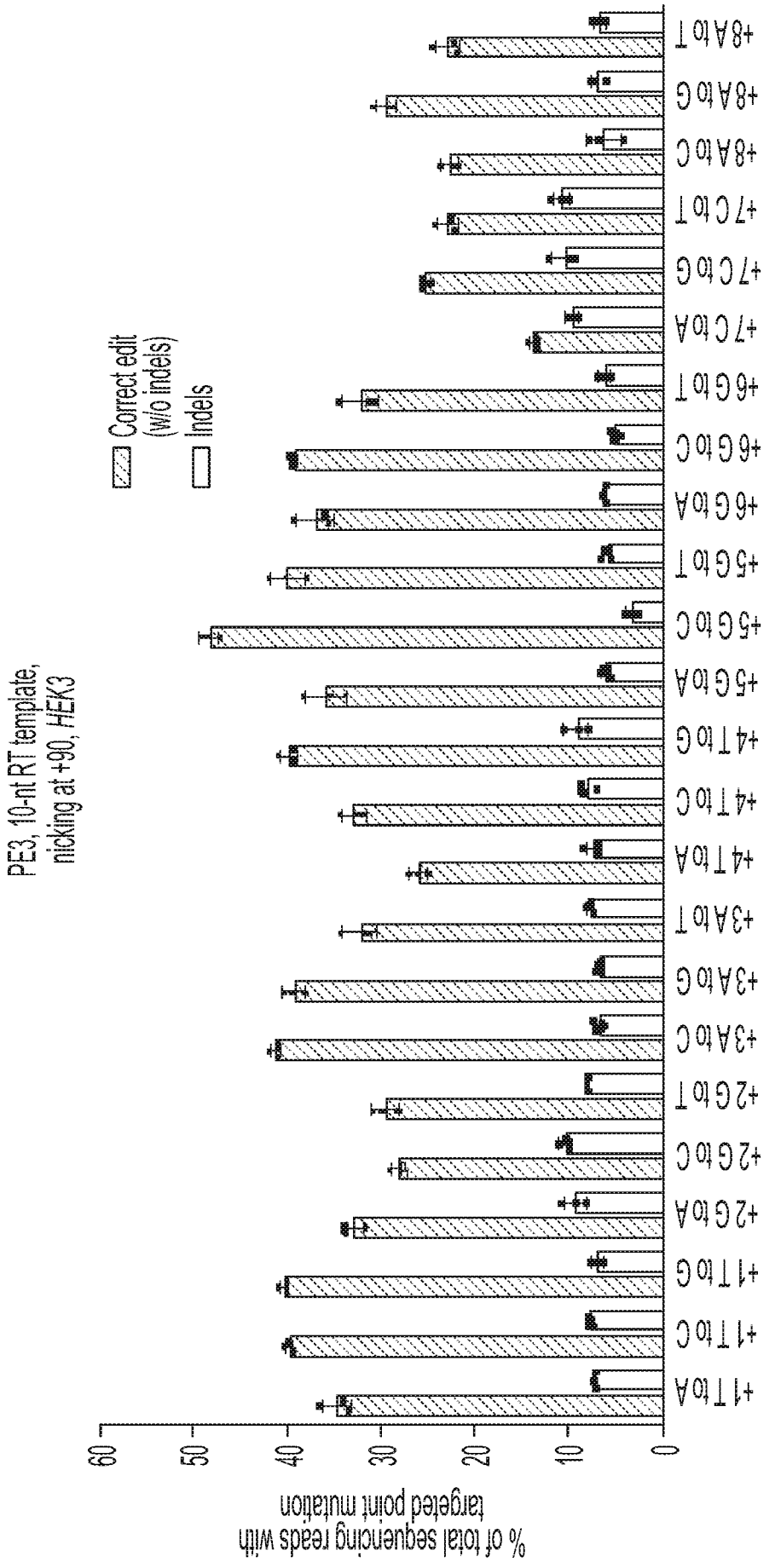

PE3, 10-nt RT template, nicking at +90, HEK3
Correct edit (w/o indels)
Indels
% of total sequencing reads with targeted point mutation
0
10
20
30
40
50
60
+1T to A
+1T to C
+1T to G
+2G to A
+2G to C
+2G to T
+3A to C
+3A to G
+3A to T
+4T to A
+4T to C
+4T to G
+5G to A
+5G to C
+5G to T
+6G to A
+6G to C
+6G to T
+7C to A
+7C to G
+7C to T
+8A to C
+8A to G
+8A to T

FIG. 41A

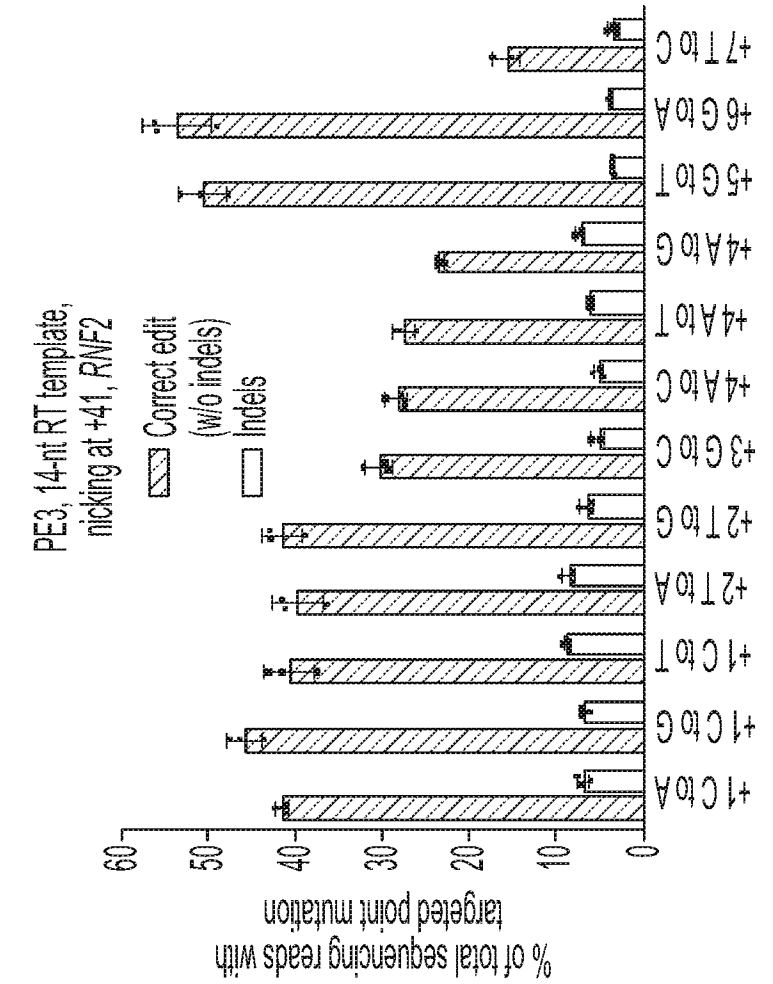


FIG. 41C

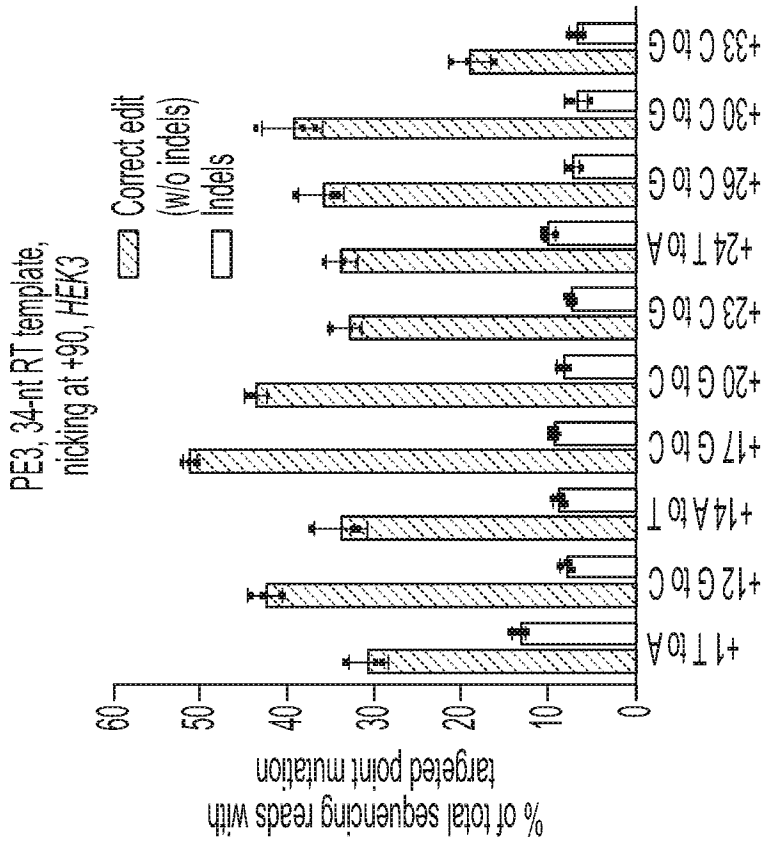


FIG. 41B

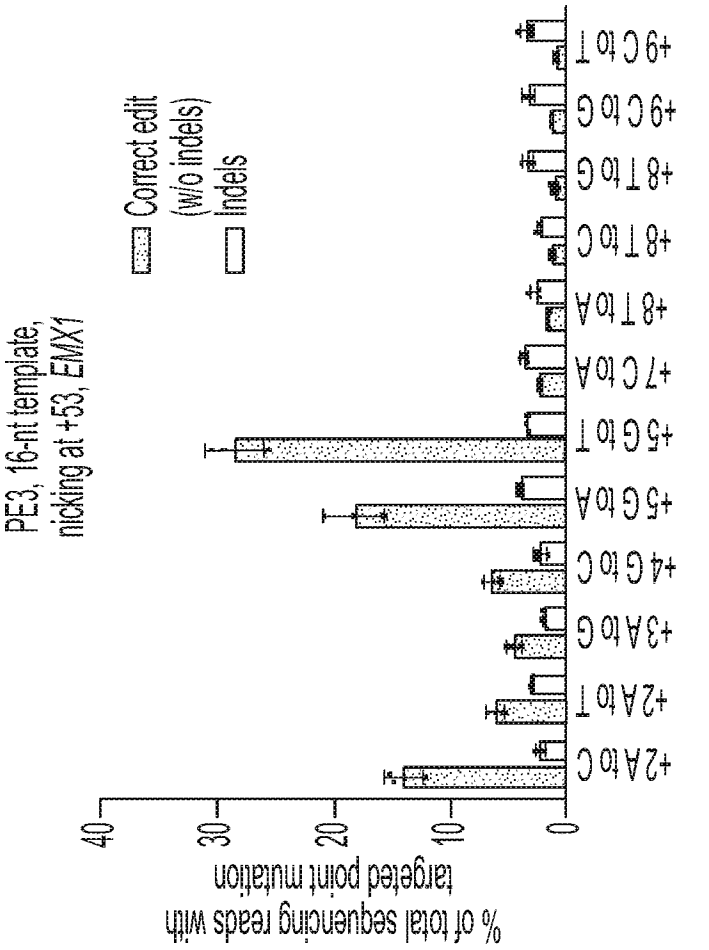


FIG. 41E

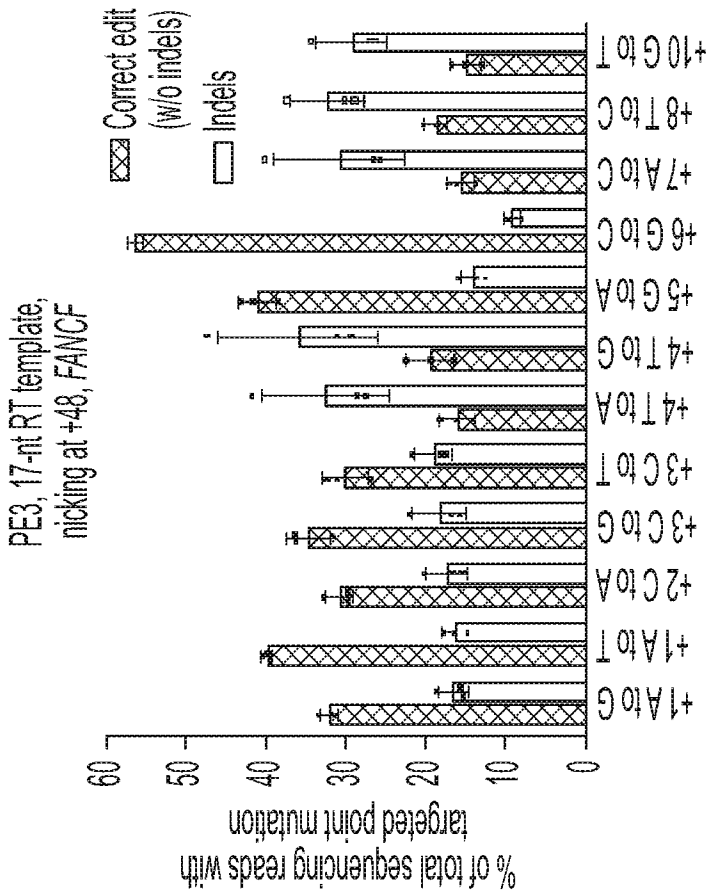


FIG. 41D

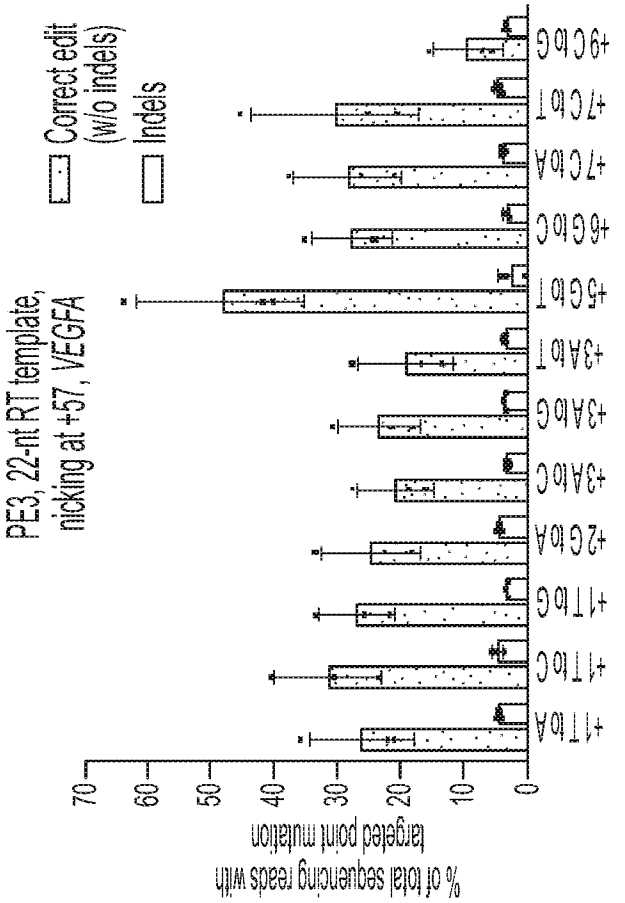


FIG. 41G

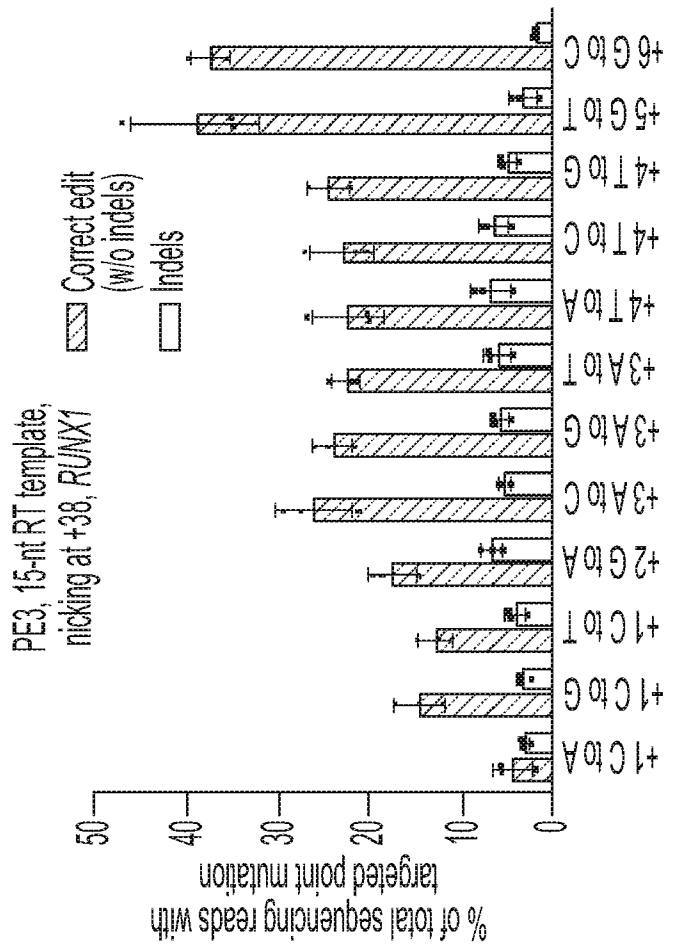


FIG. 41F

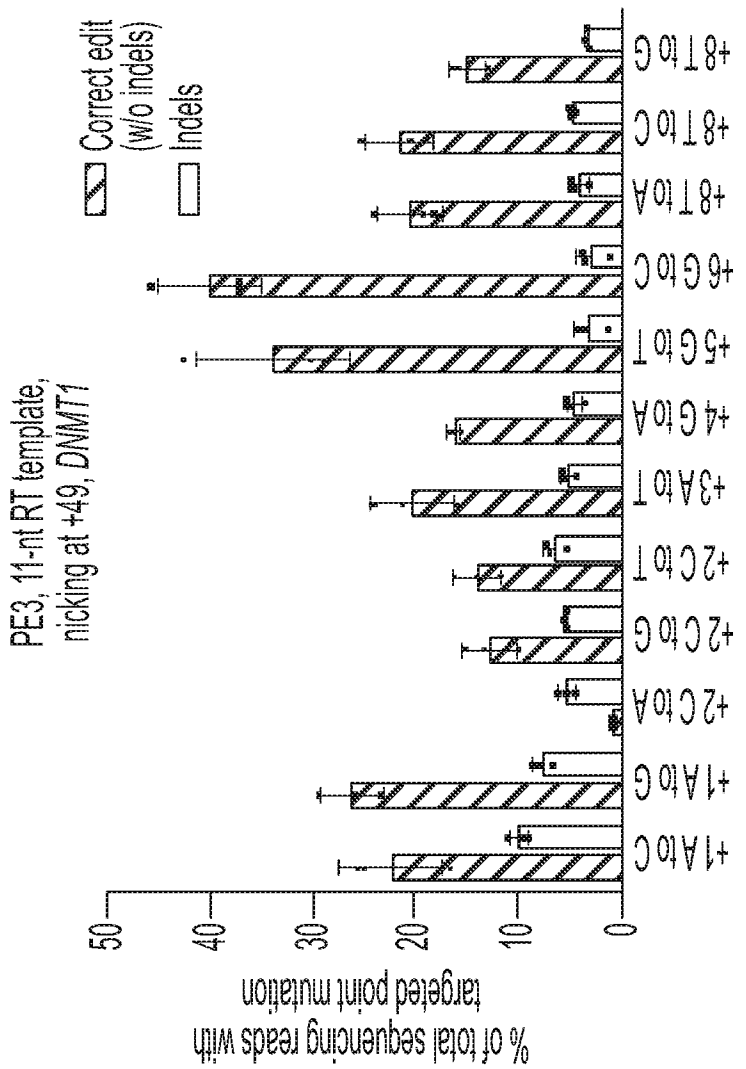

PE3, 11-nt RT template, nicking at +49, DNMT1
Correct edit (w/o indels)
Indels
% of total sequencing reads with targeted point mutation
0
10
20
30
40
50
+1A to C
+1A to G
+2C to A
+2C to G
+2C to T
+3A to T
+4G to A
+5G to T
+6G to C
+8T to A
+8T to C
+8T to G

FIG. 41H

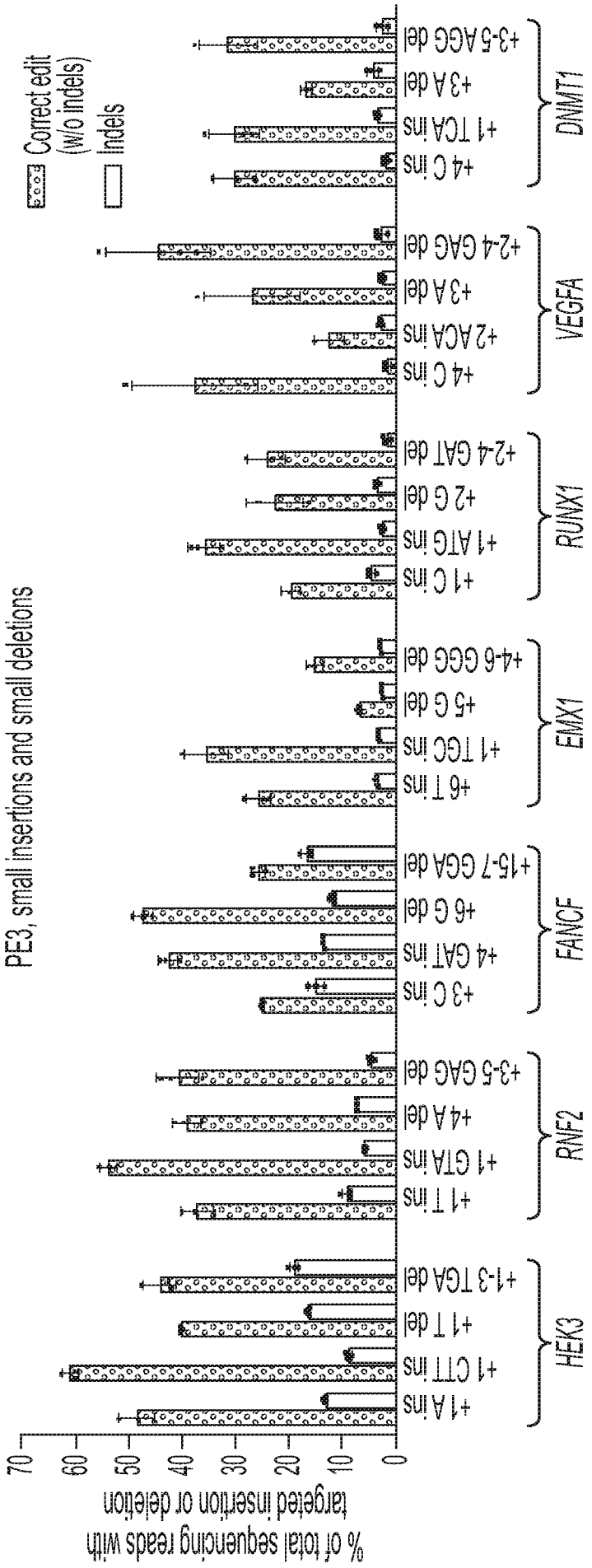

PE3, small insertions and small deletions
Correct edit (w/o indels)
Indels
% of total sequencing reads with targeted insertion or deletion
0
10
20
30
40
50
60
70
HEK3
+1 A ins
+1 CTT ins
+1 T del
+1-3 TGA del
RNF2
+1 T ins
+1 GTA ins
+4 A del
+3-5 GAG del
FANCF
+3 C ins
+4 GAT ins
+6 G del
+15-7 GGA del
EMX1
+6 T ins
+1 TGC ins
+5 G del
+4-6 GGG del
RUNX1
+1 C ins
+1 ATG ins
+2 G del
+2-4 GAT del
VEGFA
+4 C ins
+2 ACA ins
+3 A del
+2-4 GAG del
DNMT1
+4 C ins
+1 TCA ins
+3 A del
+3-5 AGG del

FIG. 41I

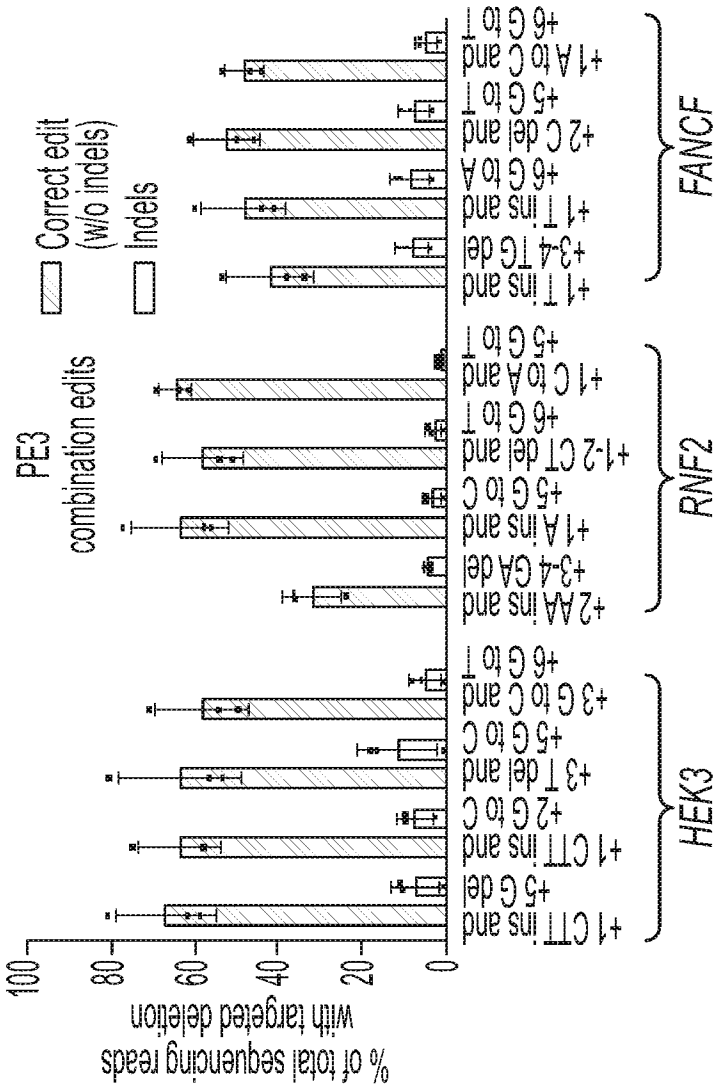


FIG. 41K

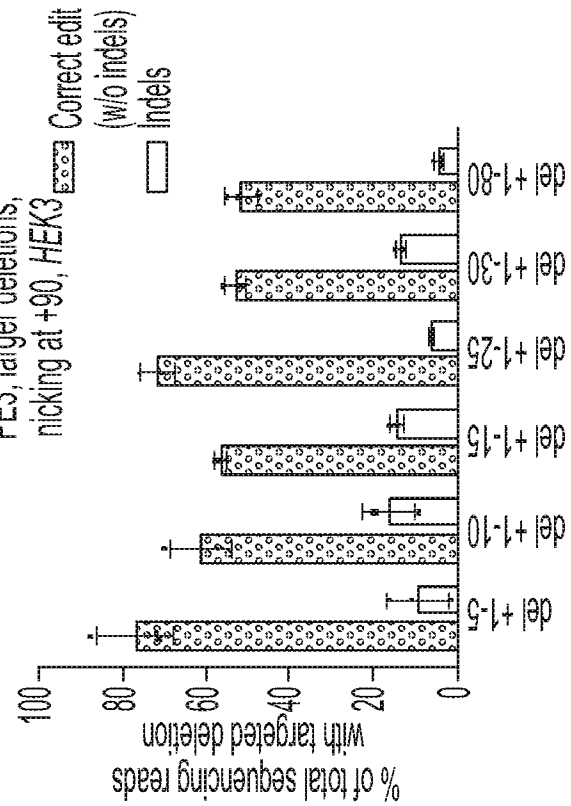


FIG. 41J

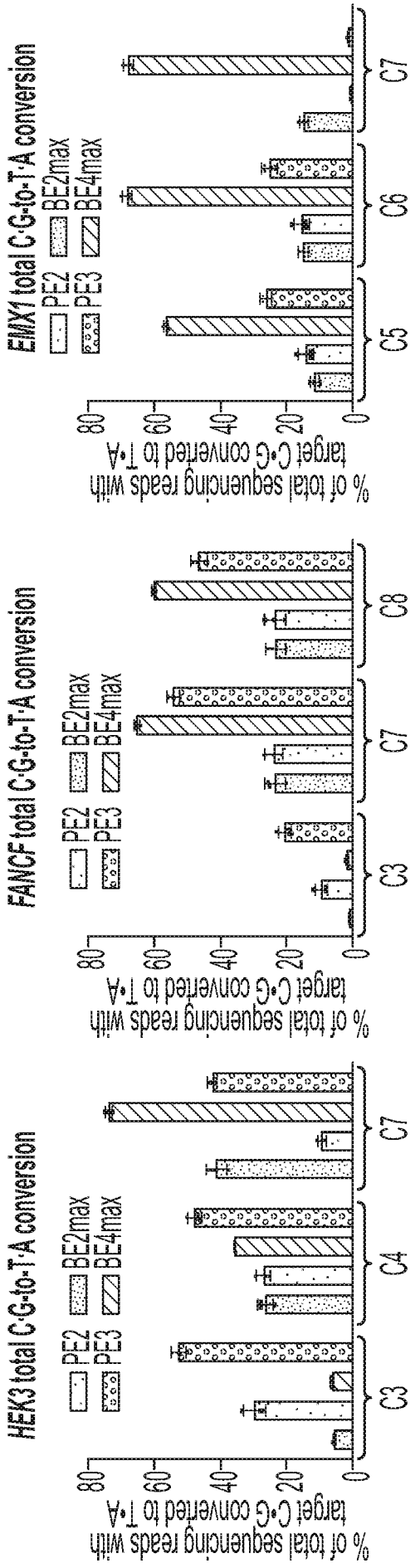


FIG. 42A

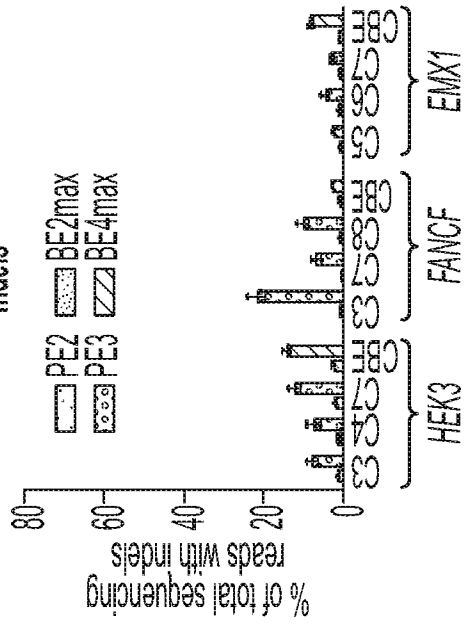


FIG. 42B

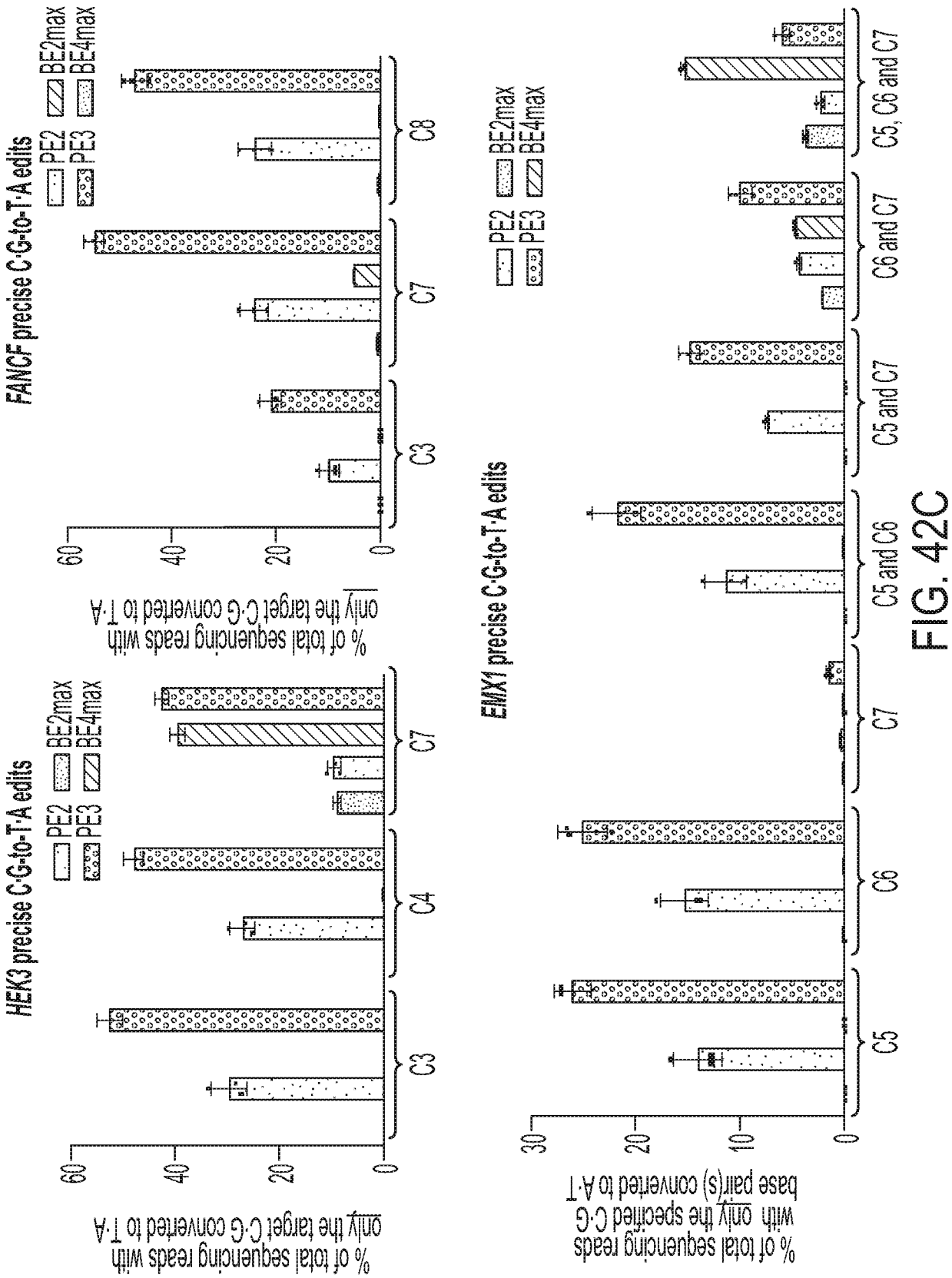

HEK3 precise C·G-to-T·A edits
PE2
PE3
BE2max
BE4max
% of total sequencing reads with only the target C·G converted to T·A
C3
C4
C7
FANCF precise C·G-to-T·A edits
C8
EMX1 precise C·G-to-T·A edits
% of total sequencing reads with only the specified C·G base pair(s) converted to A·T
C5
C6
C5 and C6
C5 and C7
C6 and C7
C5, C6 and C7

FIG. 42C

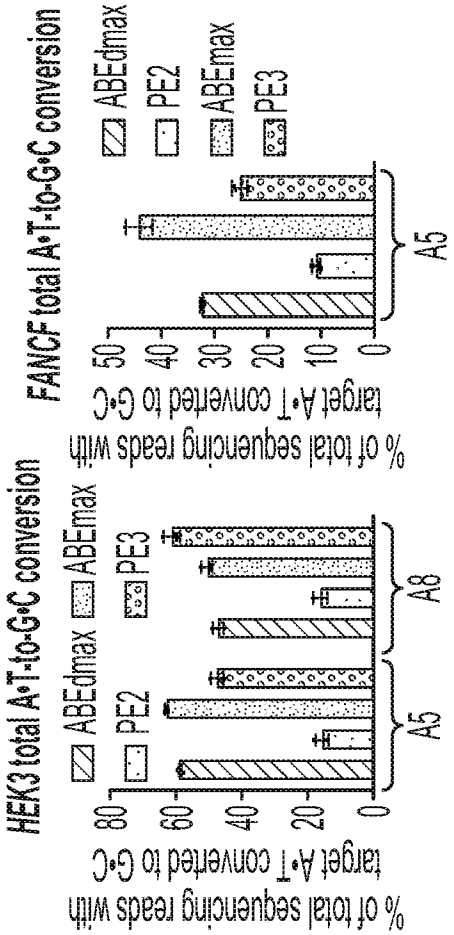


FIG. 42D

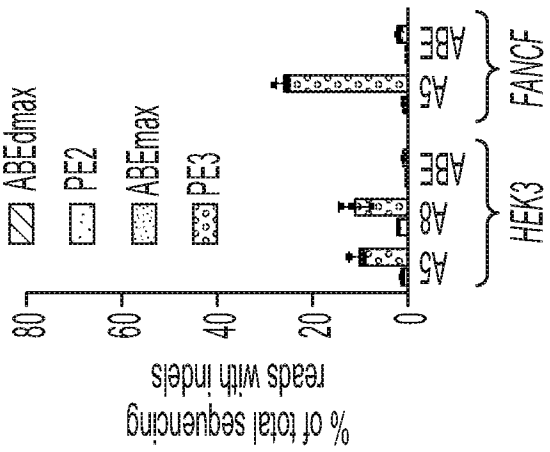


FIG. 42F

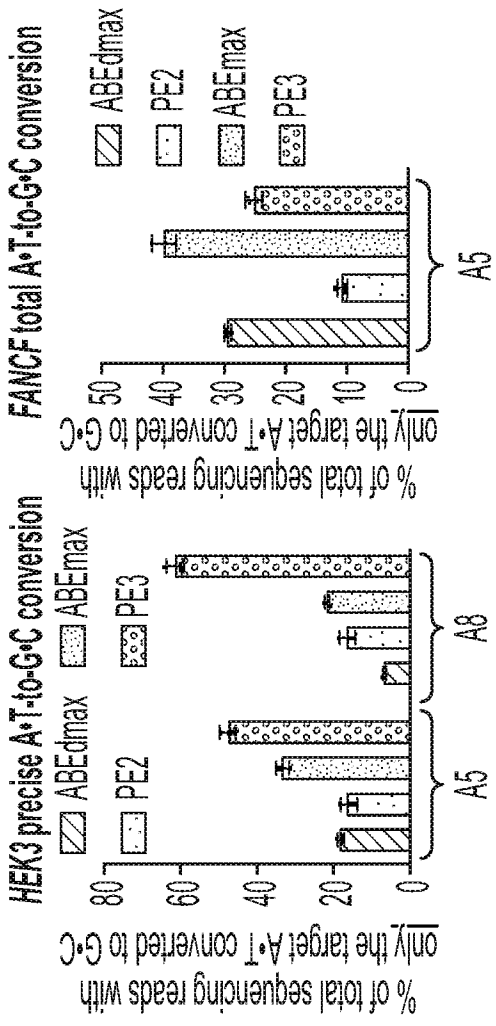


FIG. 42E

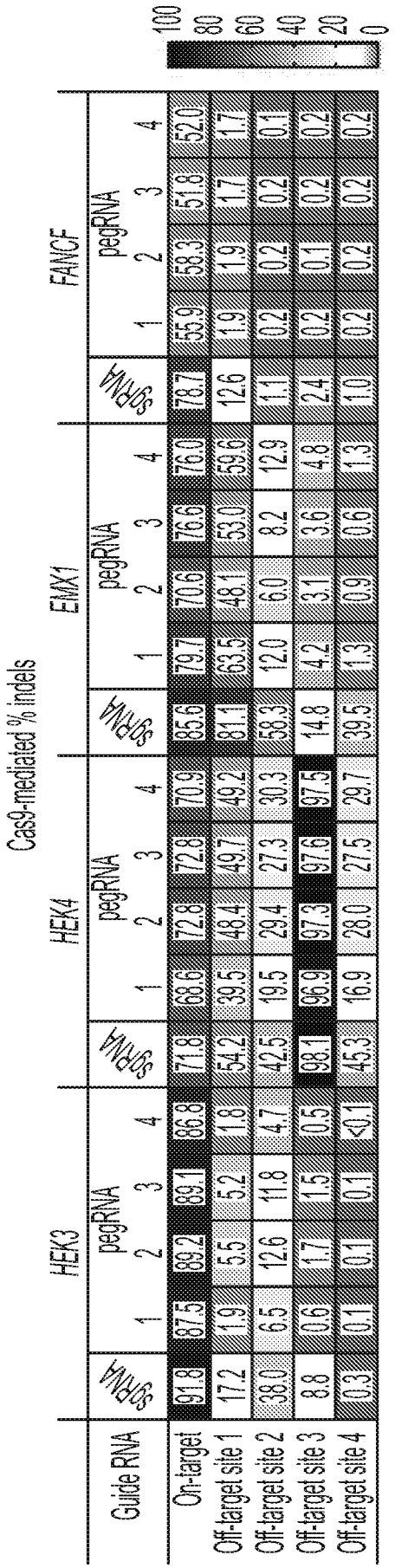

Cas9-mediated % indels
100
80
60
40
20
0
HEK3
HEK4
EMX1
FANCF
sgRNA
pegRNA
1
2
3
4
Guide RNA
On-target
Off-target site 1
Off-target site 2
Off-target site 3
Off-target site 4
91.8 87.5 89.2 89.1 86.8 71.8 68.6 72.8 72.8 70.9 85.6 79.7 70.6 76.6 76.0 78.7 55.9 58.3 51.8 52.0
17.2 1.9 5.5 5.2 1.8 54.2 39.5 48.4 49.7 49.2 81.1 63.5 48.1 53.0 59.6 12.6 1.9 1.9 1.7 1.7
38.0 6.5 12.6 11.8 4.7 42.5 19.5 29.4 27.3 30.3 58.3 12.0 6.0 8.2 12.9 1.1 0.2 0.2 0.2 0.1
8.8 0.6 1.7 1.5 0.5 98.1 96.9 97.3 97.6 97.5 14.8 4.2 3.1 3.6 4.8 2.4 0.2 0.1 0.2 0.2
0.3 0.1 0.1 0.1 <0.1 45.3 16.9 28.0 27.5 29.7 39.5 1.3 0.9 0.6 1.3 1.0 0.2 0.2 0.2 0.2

FIG. 42G

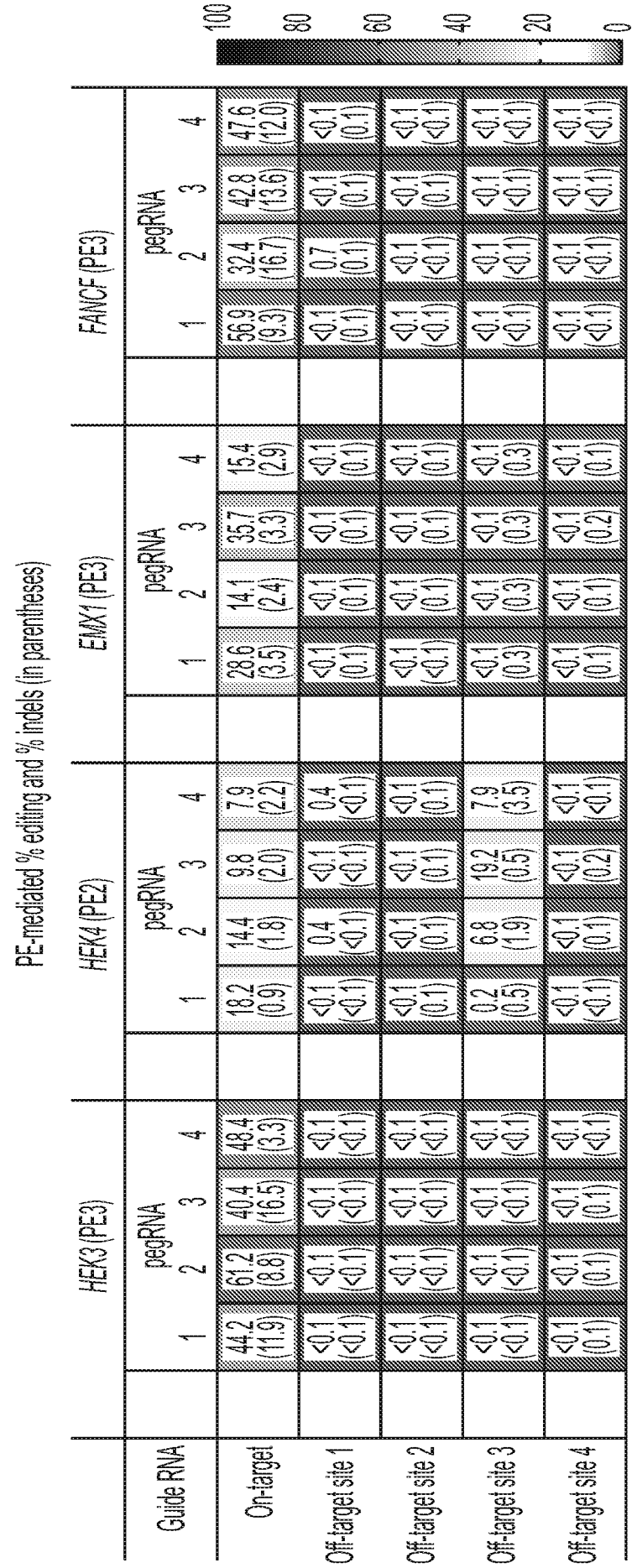

PE-mediated % editing and % indels (in parentheses)
100
80
60
40
20
0
Guide RNA
HEK3 (PE3)
HEK4 (PE2)
EMX1 (PE3)
FANCF (PE3)
pegRNA
1 2 3 4
On-target
Off-target site 1
Off-target site 2
Off-target site 3
Off-target site 4
44.2 (11.9)
61.2 (8.8)
40.4 (16.5)
48.4 (3.3)
18.2 (0.9)
14.4 (1.8)
9.8 (2.0)
7.9 (2.2)
28.6 (3.5)
14.1 (2.4)
35.7 (3.3)
15.4 (2.9)
56.9 (9.3)
32.4 (16.7)
42.8 (13.6)
47.6 (12.0)
<0.1 (<0.1)
0.4 (<0.1)
<0.1 (<0.1)
0.4 (<0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
0.7 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
0.2 (0.5)
6.8 (1.9)
19.2 (0.5)
7.9 (3.5)
<0.1 (0.3)
<0.1 (0.3)
<0.1 (0.3)
<0.1 (0.3)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (0.1)
<0.1 (0.2)
<0.1 (<0.1)
<0.1 (0.1)
<0.1 (0.1)
<0.1 (0.2)
<0.1 (<0.1)
<0.1 (0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)
<0.1 (<0.1)

FIG. 42H

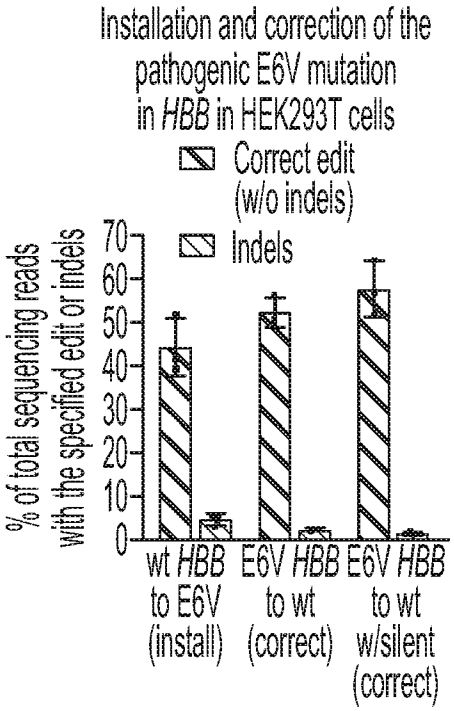


FIG. 43A

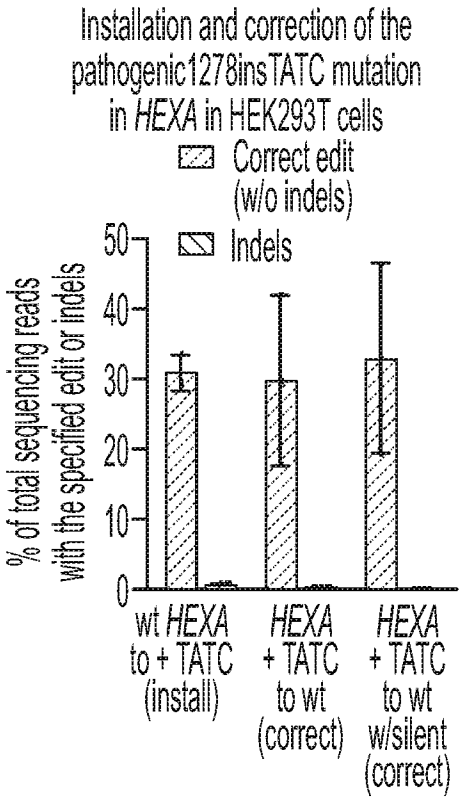


FIG. 43B

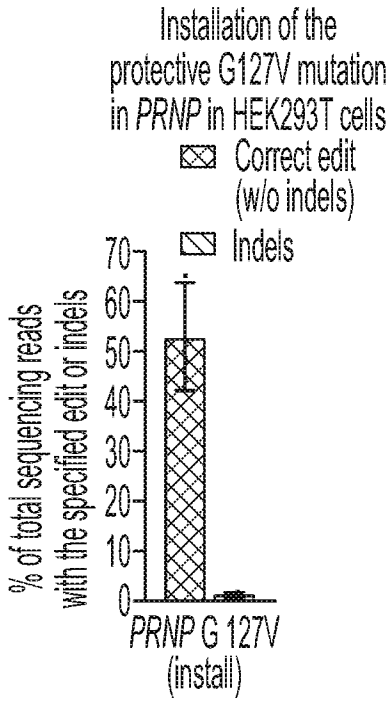


FIG. 43C

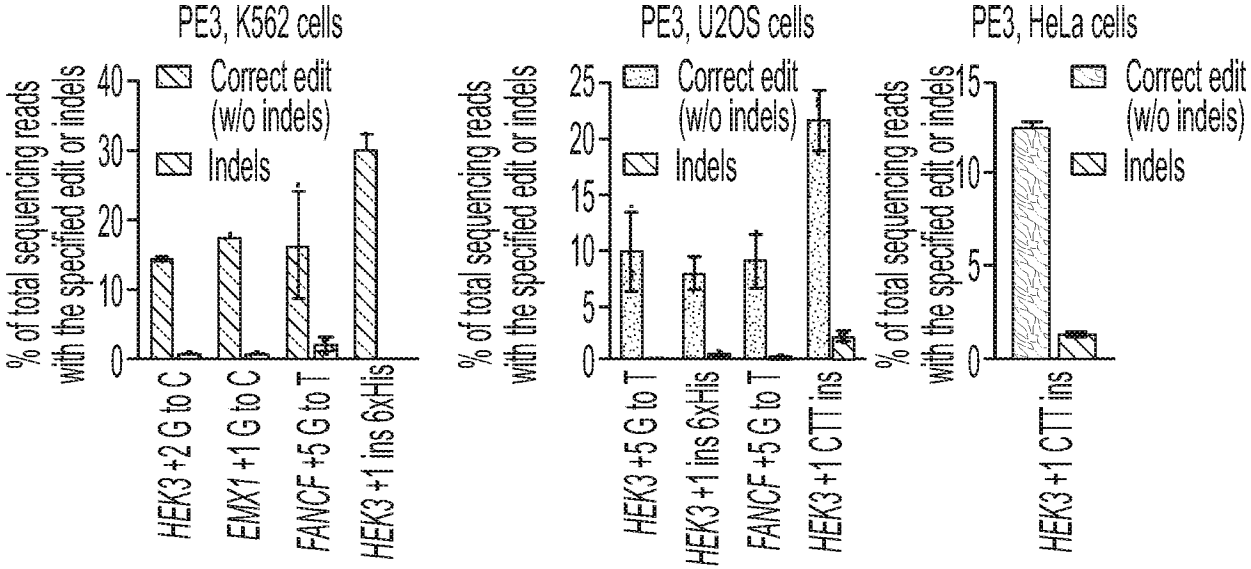


FIG. 43D

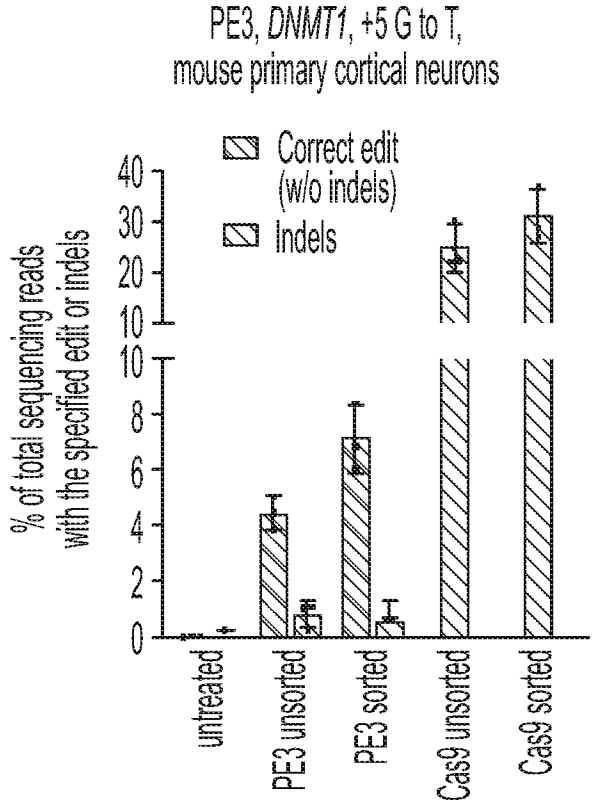


FIG. 43E

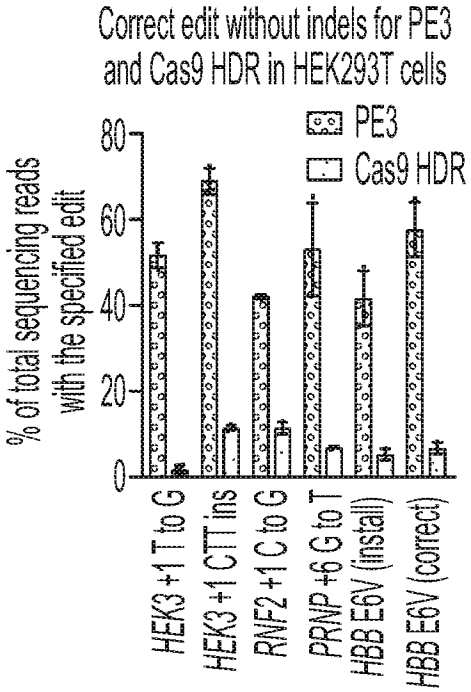


FIG. 43F

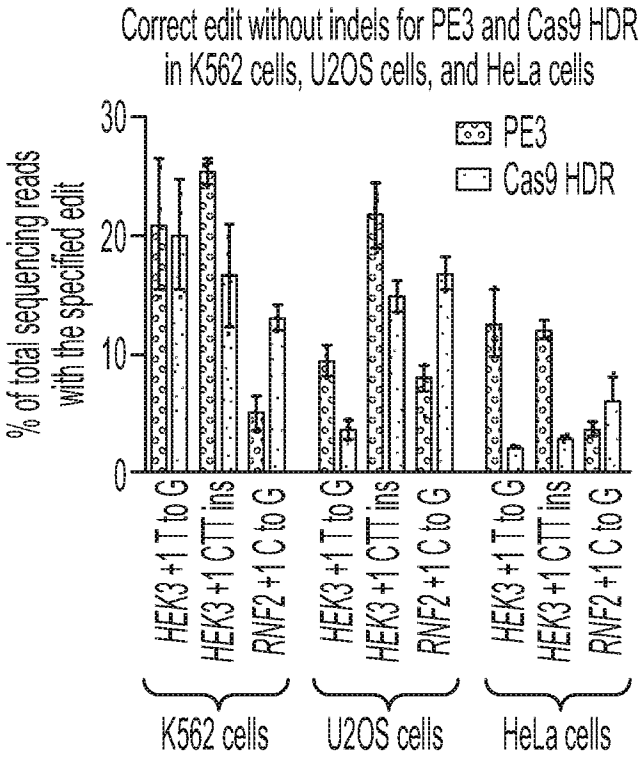


FIG. 43G

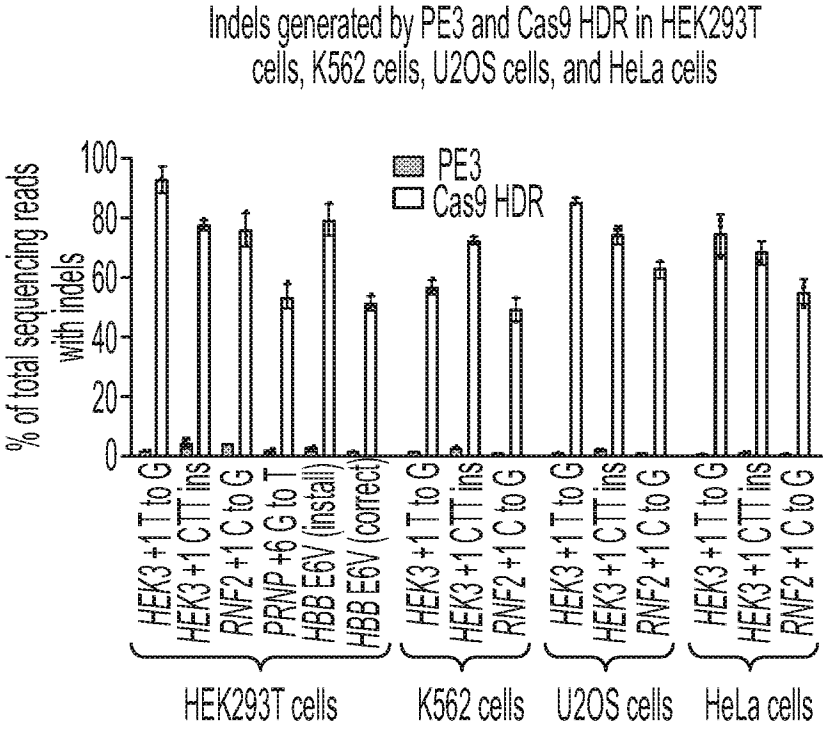


FIG. 43H

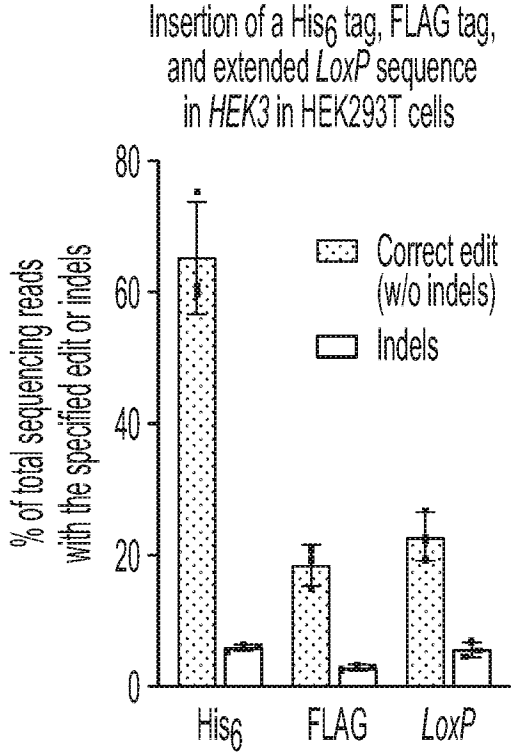


FIG. 43I

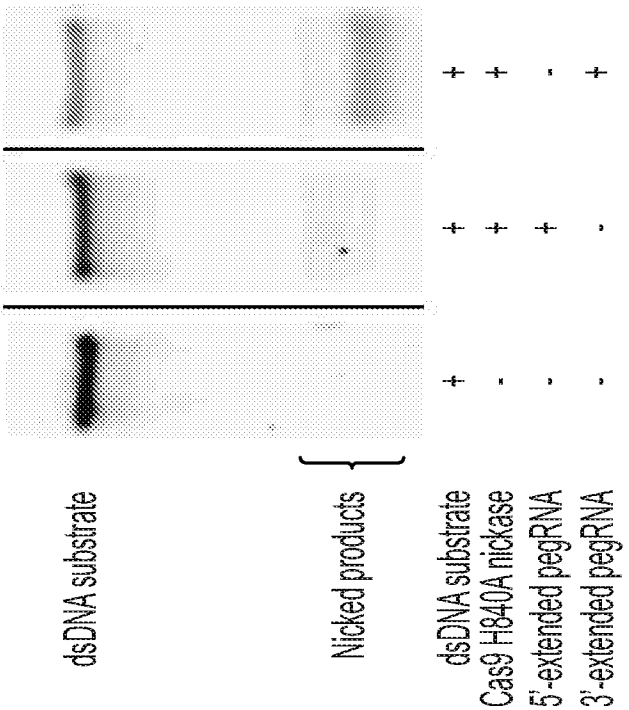


FIG. 44B

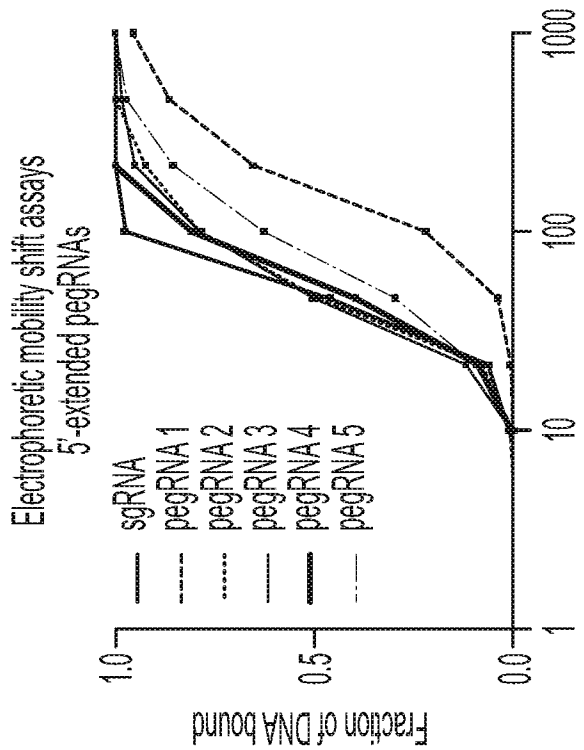


FIG. 44A

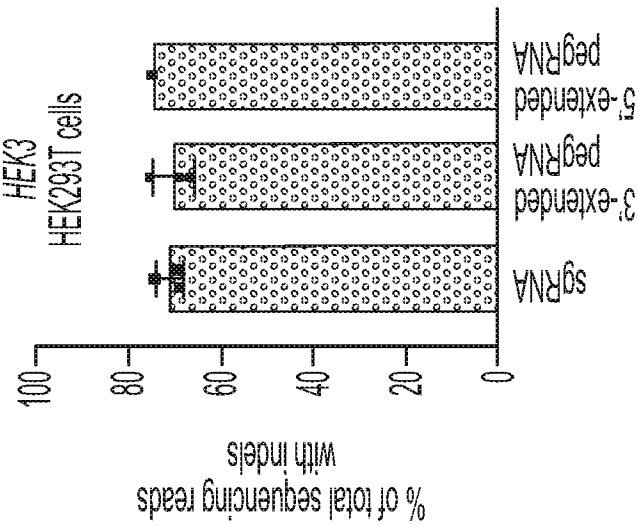

% of total sequencing reads with indels
100
80
60
40
20
0
HEK3
HEK293T cells
sgRNA
3'-extended pegRNA
5'-extended pegRNA

FIG. 44C

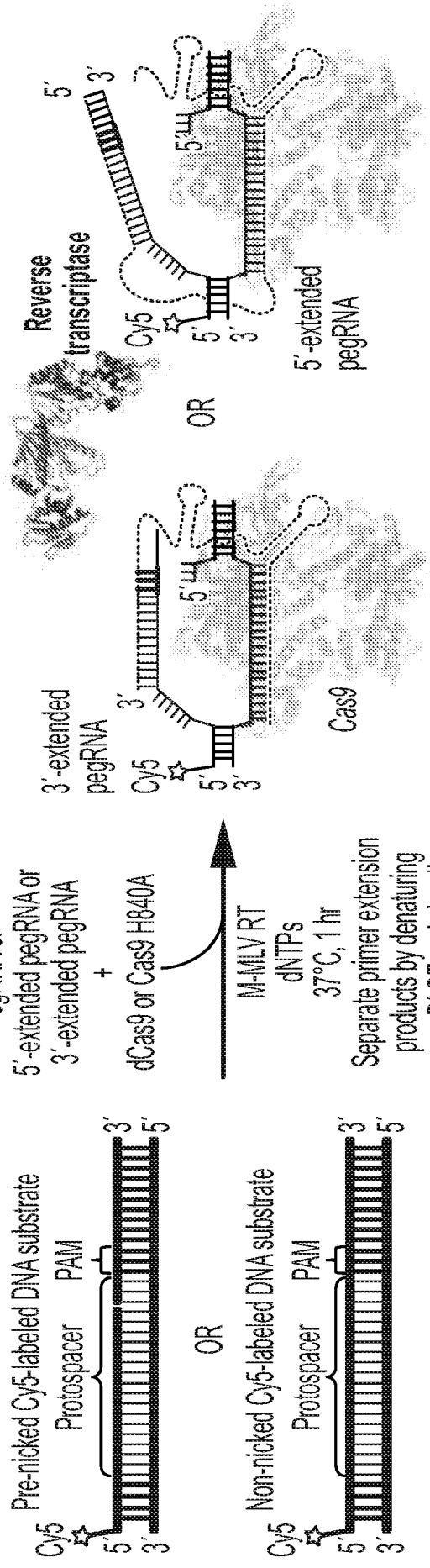

Pre-nicked Cy5-labeled DNA substrate
Protospacer
PAM
Cy5
5′
3′
OR
Non-nicked Cy5-labeled DNA substrate
Protospacer
PAM
Cy5
5′
3′
sgRNA or
5′-extended pegRNA or
3′-extended pegRNA
+
dCas9 or Cas9 H840A
M-MLV RT
dNTPs
37°C, 1 hr
Separate primer extension products by denaturing urea PAGE and visualize Cy5 fluorescence
3′-extended pegRNA
Cy5
Cas9
Reverse transcriptase
OR
5′-extended pegRNA
Cy5

FIG. 44D

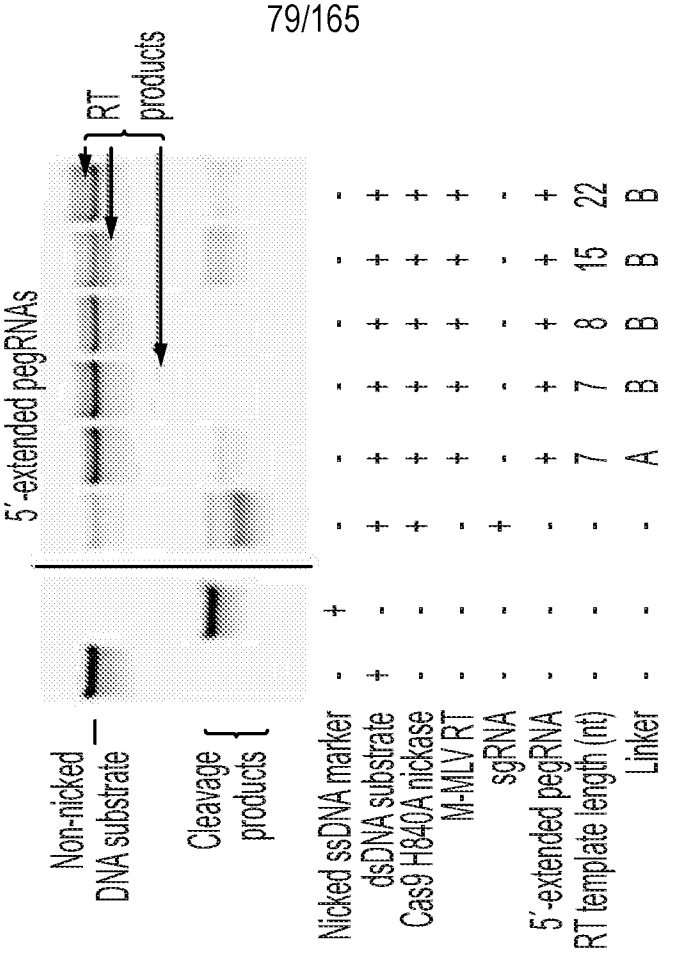


FIG. 44F

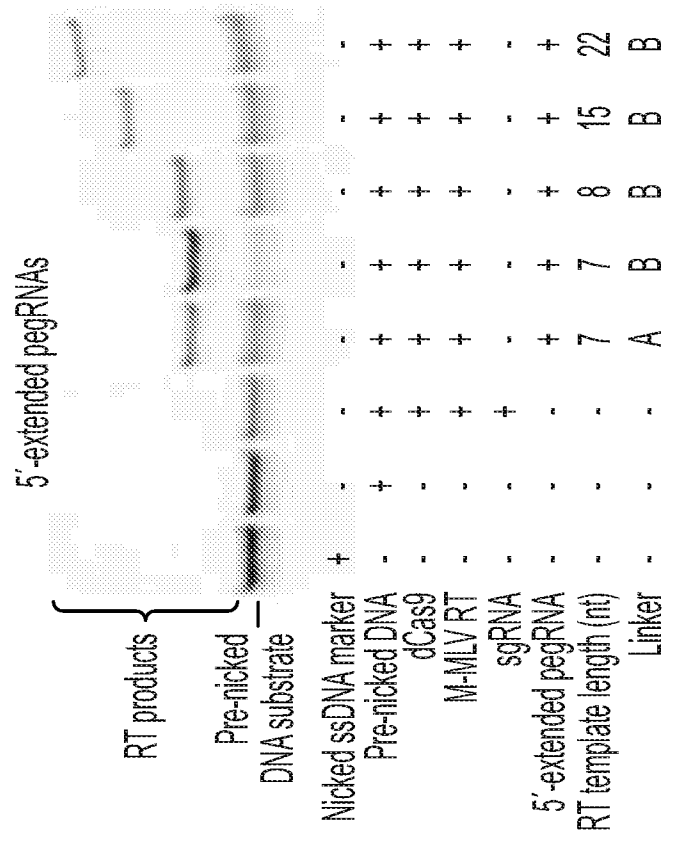


FIG. 44E

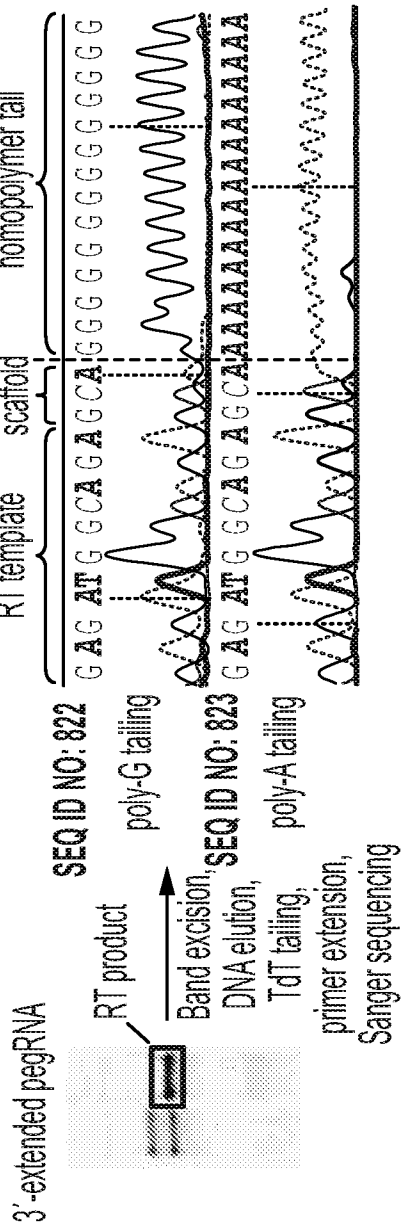


FIG. 44G

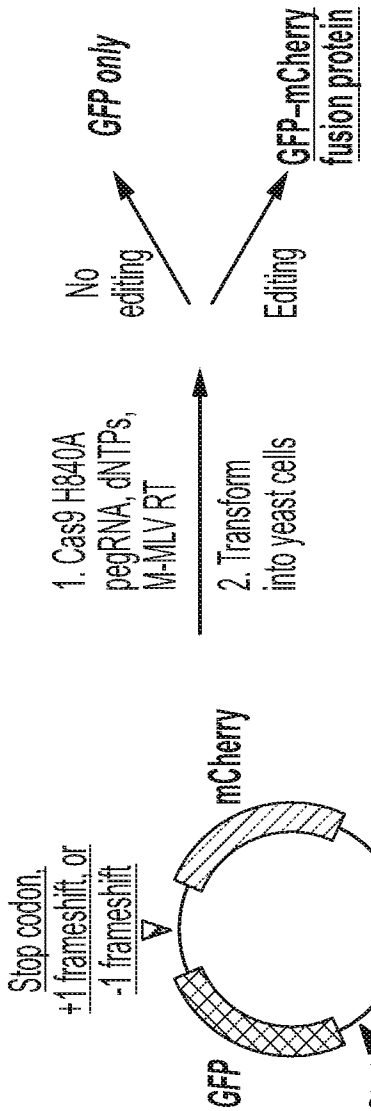


FIG. 45A

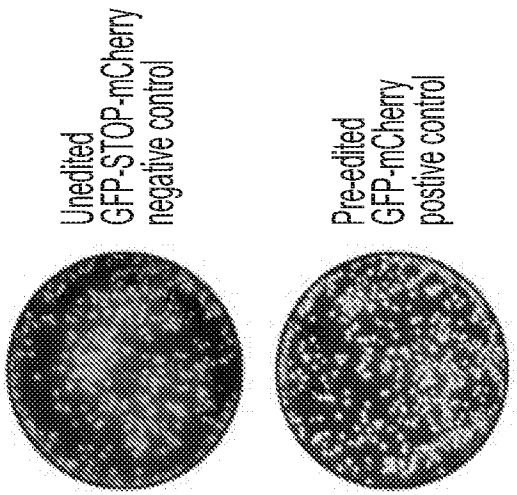

Unedited
GFP-STOP-mCherry
negative control
Pre-edited
GFP-mCherry
postive control

FIG. 45B

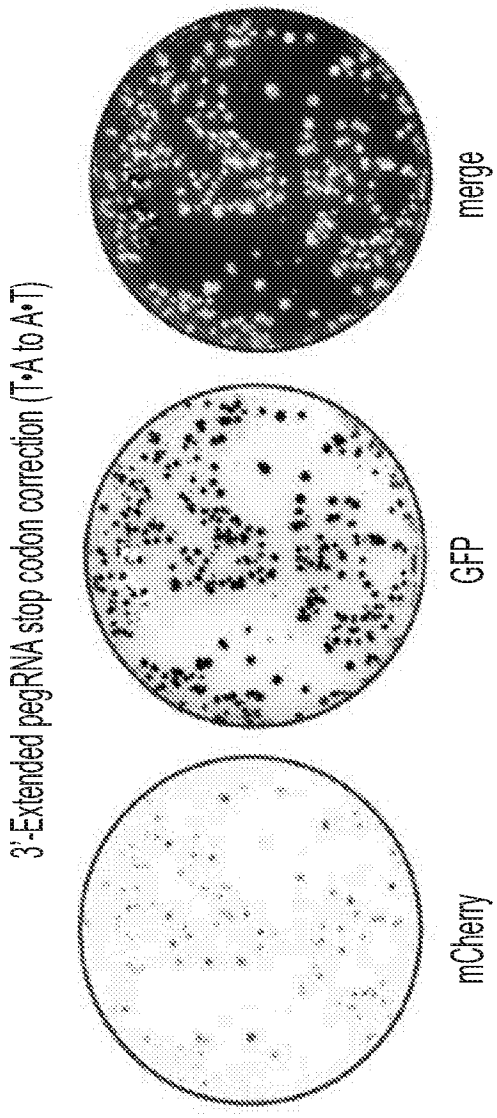


FIG. 45C

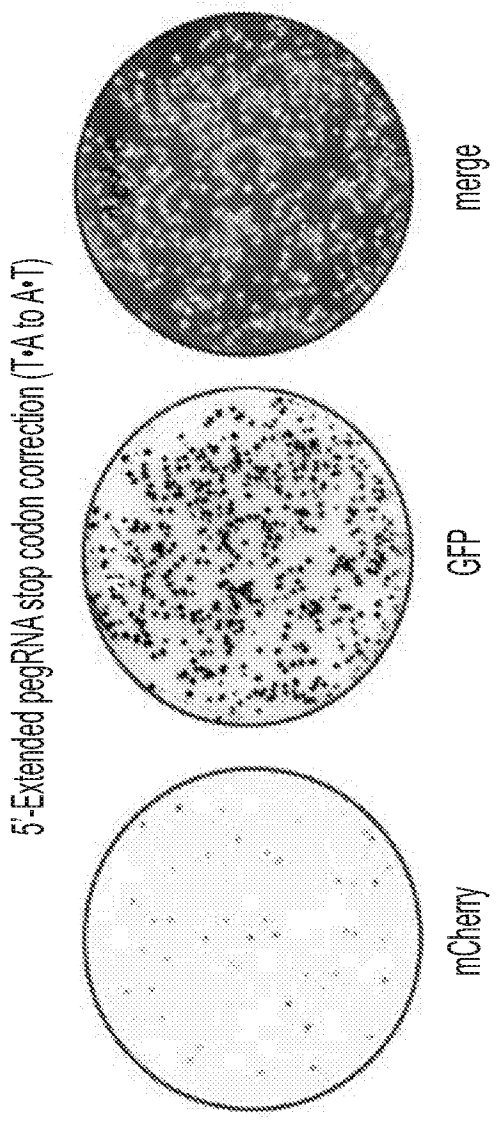


FIG. 45D

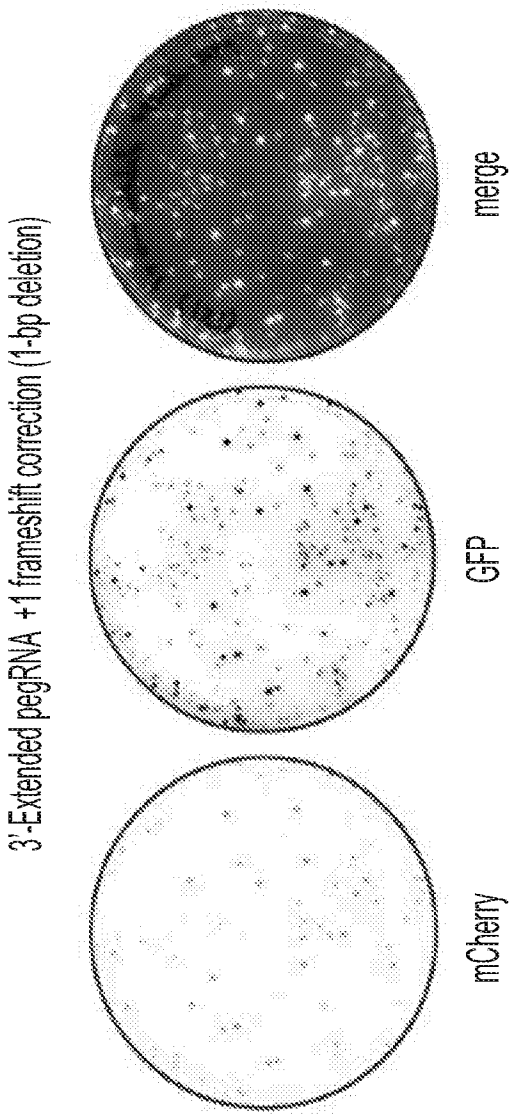


FIG. 45E

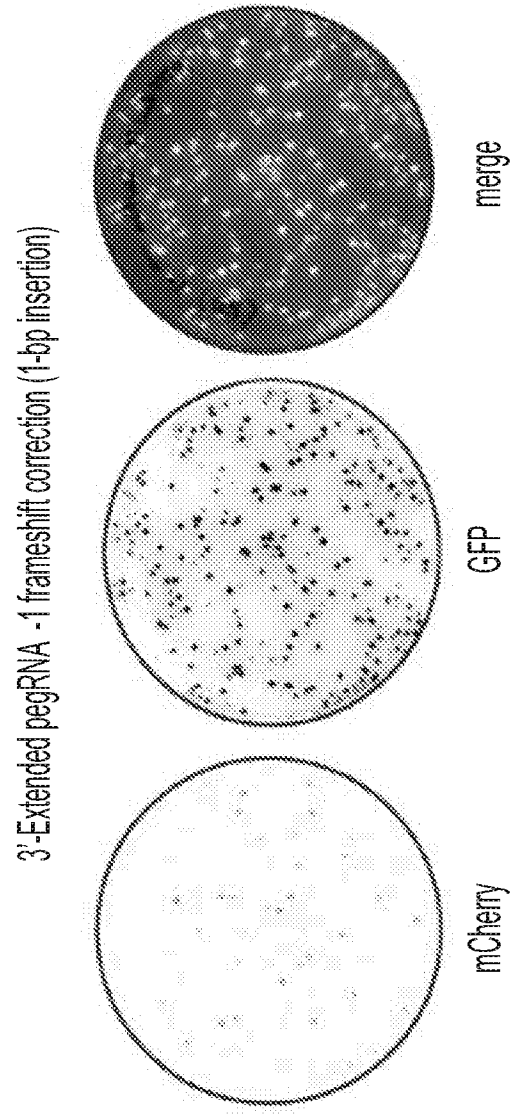


FIG. 45F

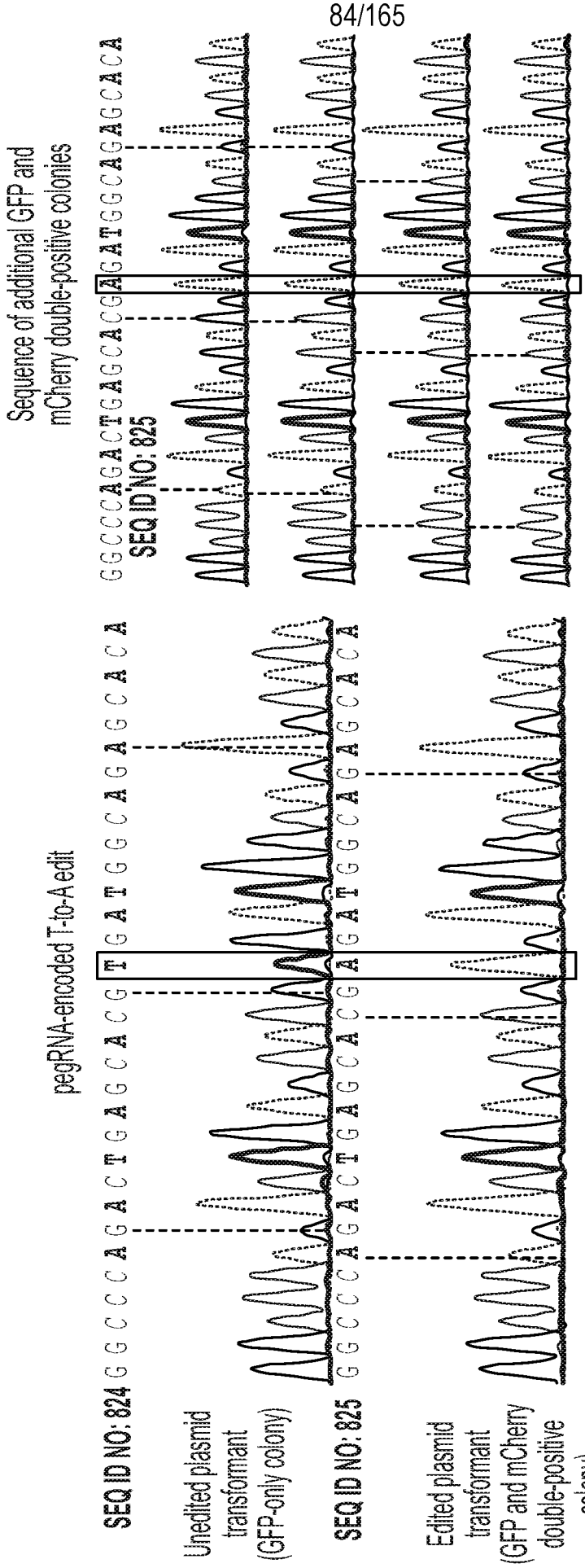

Sequence of additional GFP and mCherry double-positive colonies
SEQ ID NO: 825
G G C C C A G A C T G A G C A C G A G A T G G C A G A G C A C A
pegRNA-encoded T-to-A edit
SEQ ID NO: 824
G G C C C A G A C T G A G C A C G T G A T G G C A G A G C A C A
Unedited plasmid transformant (GFP-only colony)
SEQ ID NO: 825
G G C C C A G A C T G A G C A C G A G A T G G C A G A G C A C A
Edited plasmid transformant (GFP and mCherry double-positive colony)

FIG. 45G

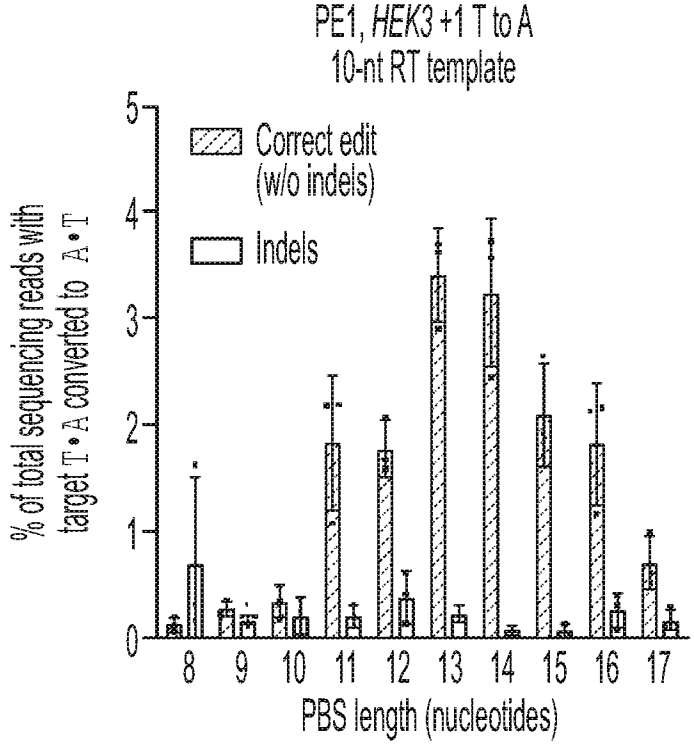

PE1, HEK3 +1 T to A
10-nt RT template
Correct edit
(w/o indels)
Indels
% of total sequencing reads with
target T • A converted to A • T
0
1
2
3
4
5
8
9
10
11
12
13
14
15
16
17
PBS length (nucleotides)

FIG. 46A

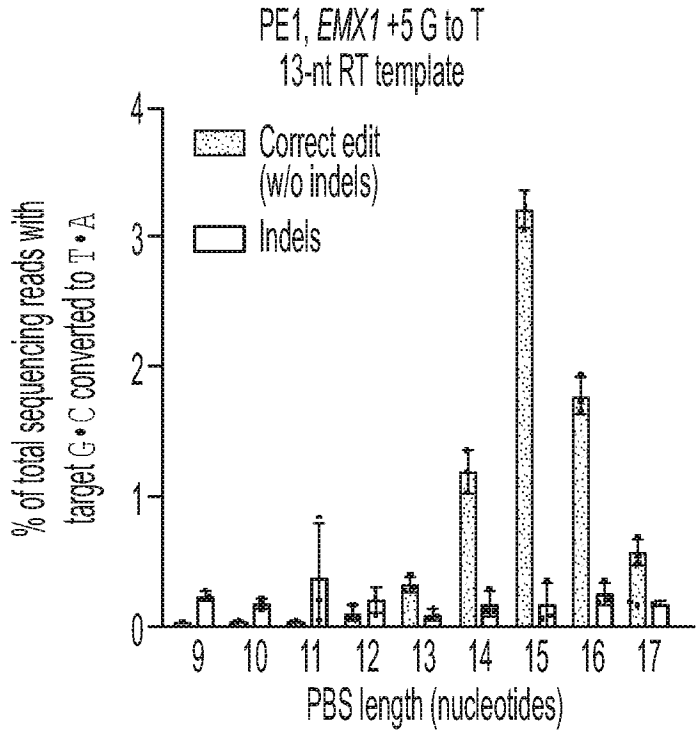


FIG. 46B

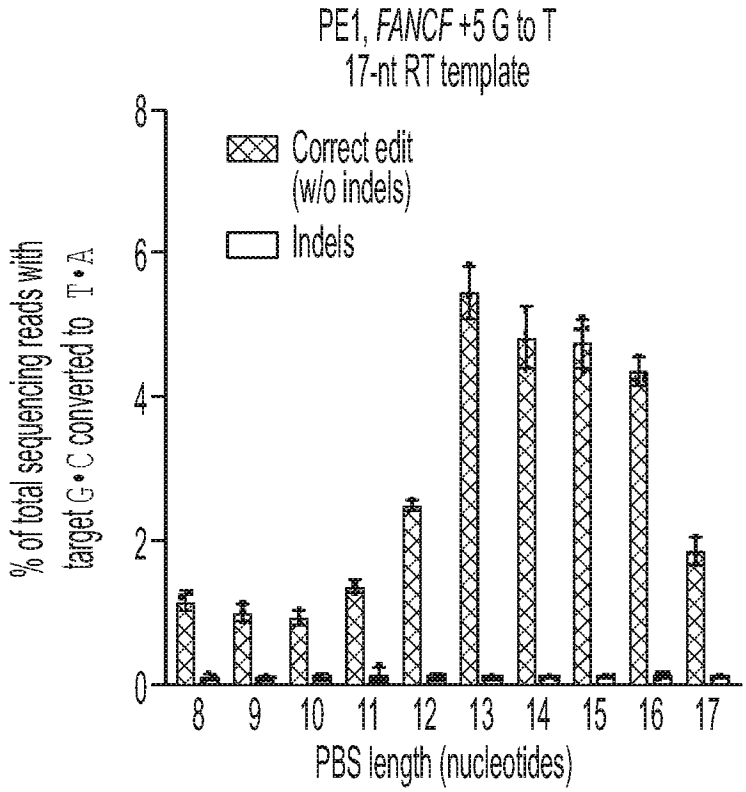


FIG. 46C

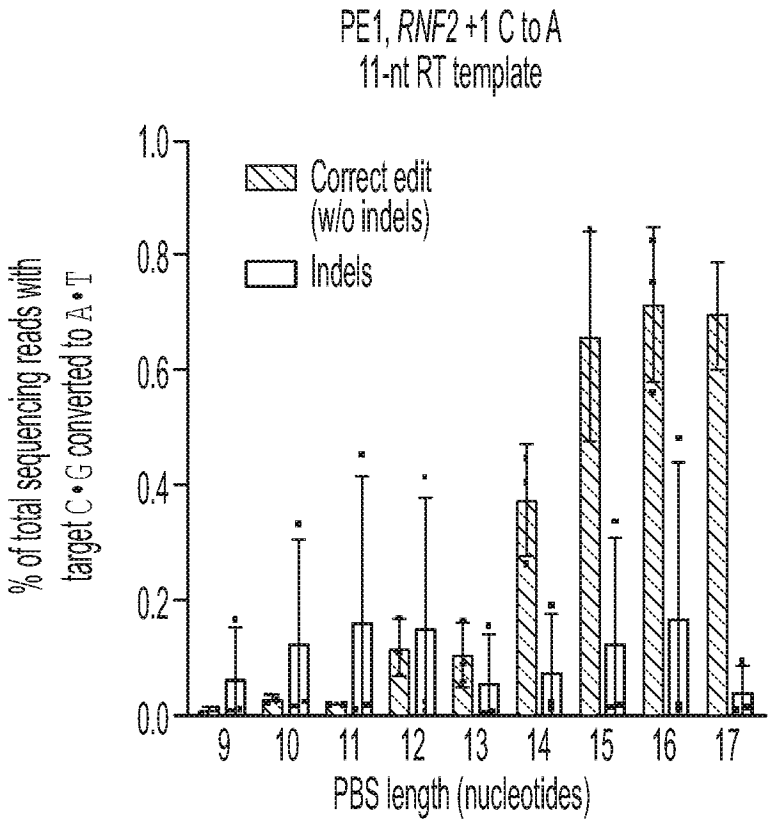


FIG. 46D

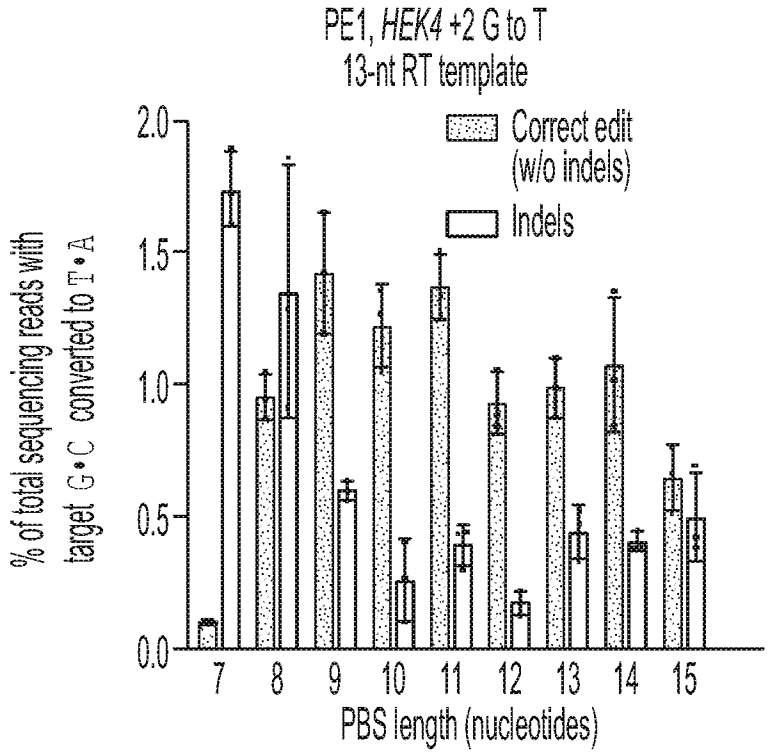


FIG. 46E

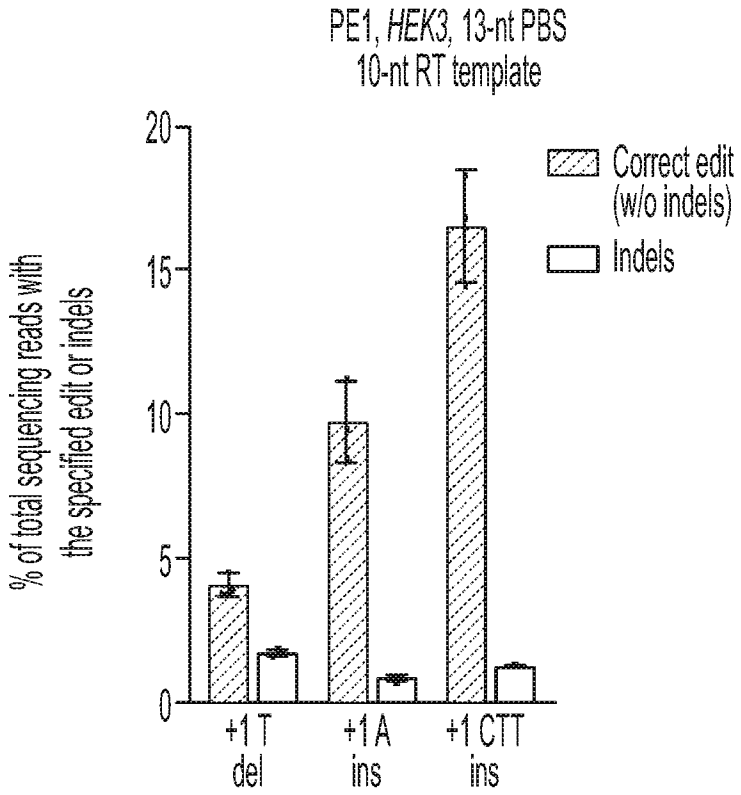


FIG. 46F

PE1	Cas9 H840A-M-MLV RT (wt)
PE1-M1	Cas9 H840A-M-MLV RT (D200N)
PE1-M2	Cas9 H840A-M-MLV RT (D200N+L603W)
PE1-M3	Cas9 H840A-M-MLV RT (D200N+L603W+T330P)
PE1-M6	Cas9 H840A-M-MLV RT (D200N+L603W+T330P+D524G+E562Q+D583N)
PE1-M15	Cas9 H840A-M-MLV RT (P51L+S67R+E67K+T197A+H204R+E302K+F309N+W1331F+T330P+L345G+N454K+D524G+D583N+H594Q+D653N)
PE1-M3inv	M-MLV RT (D200N+L603W+T330P)-Cas9 H840A
PE2	Cas9 H840A-M-MLV RT (D200N+L603W+T330P+T306K+W313F)

FIG. 47A

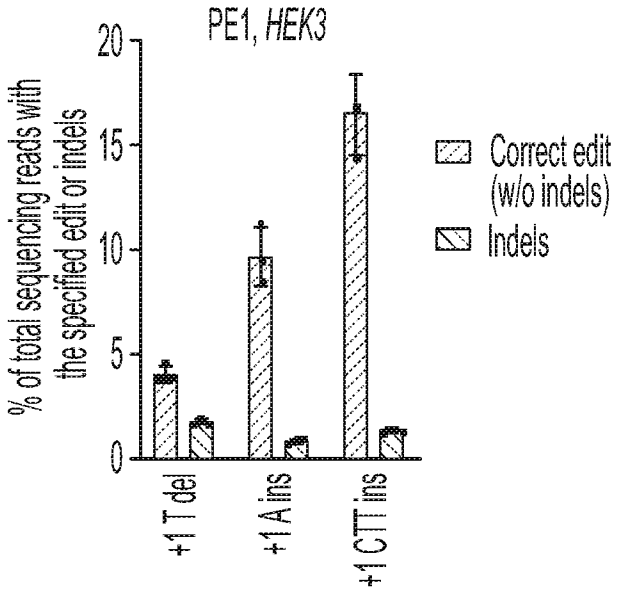


FIG. 47B

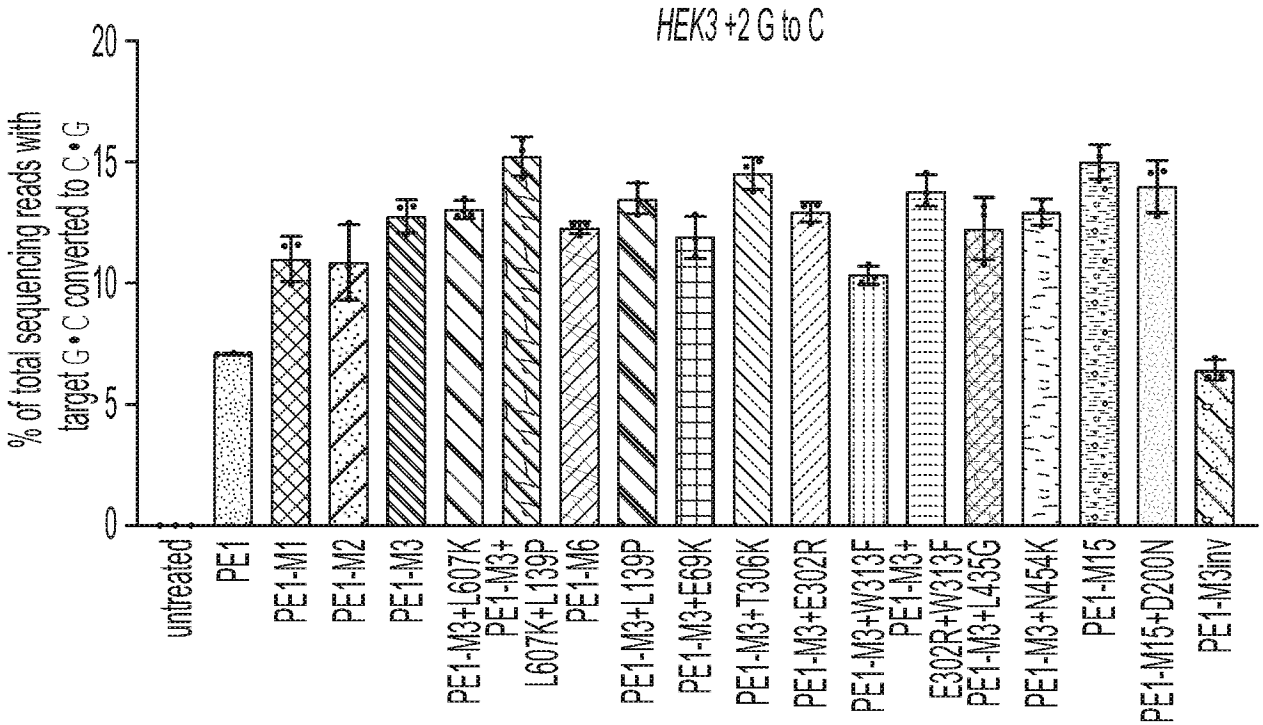


FIG. 47C

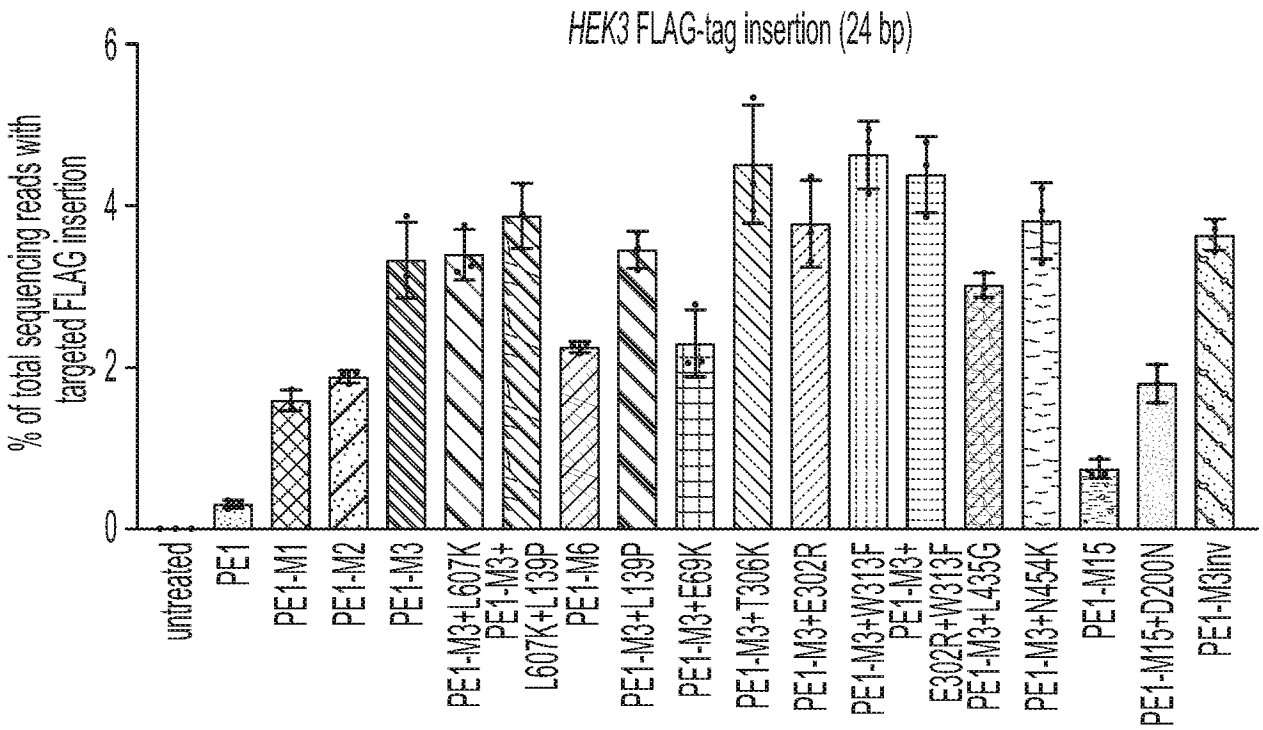


FIG. 47D

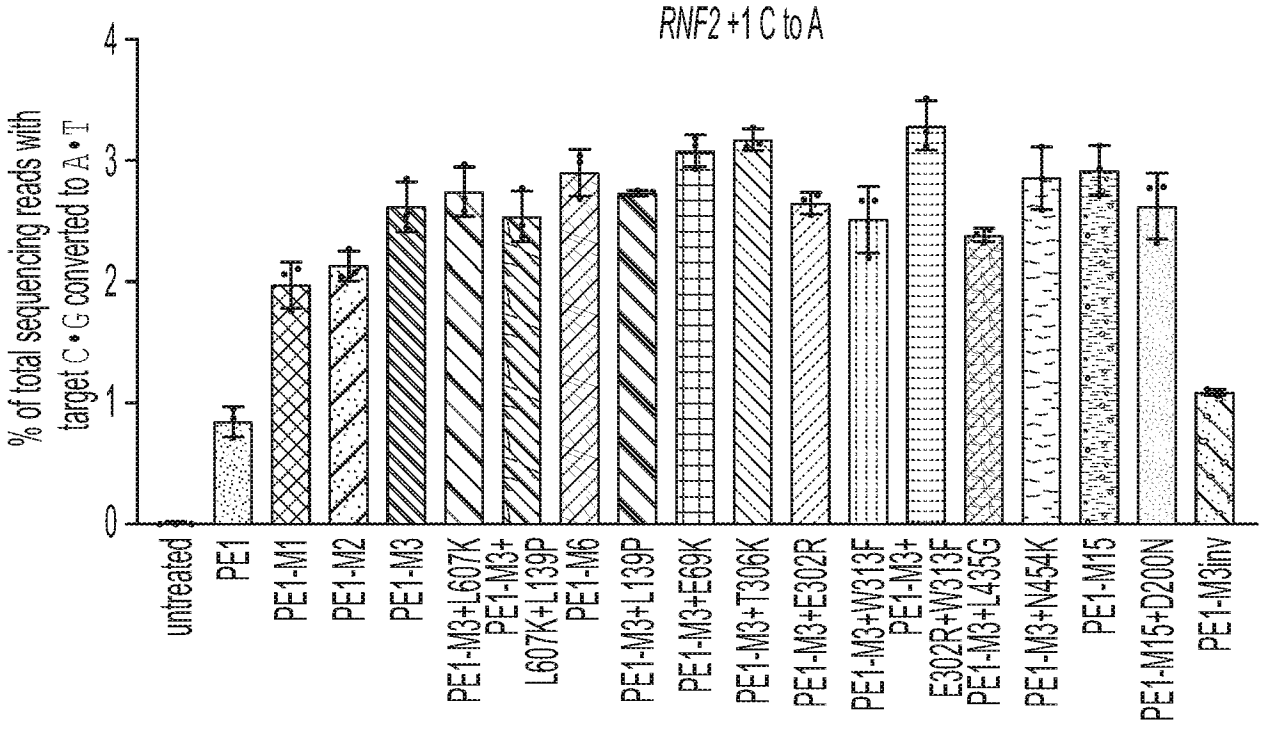


FIG. 47E

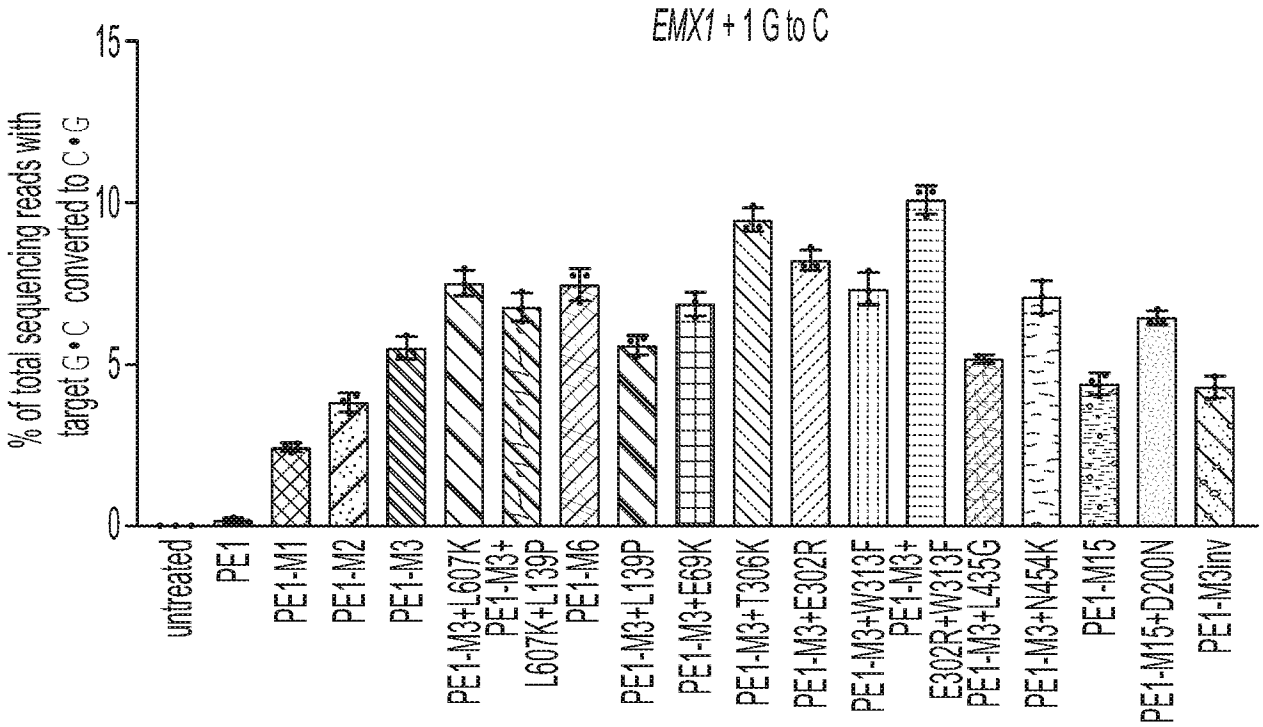


FIG. 47F

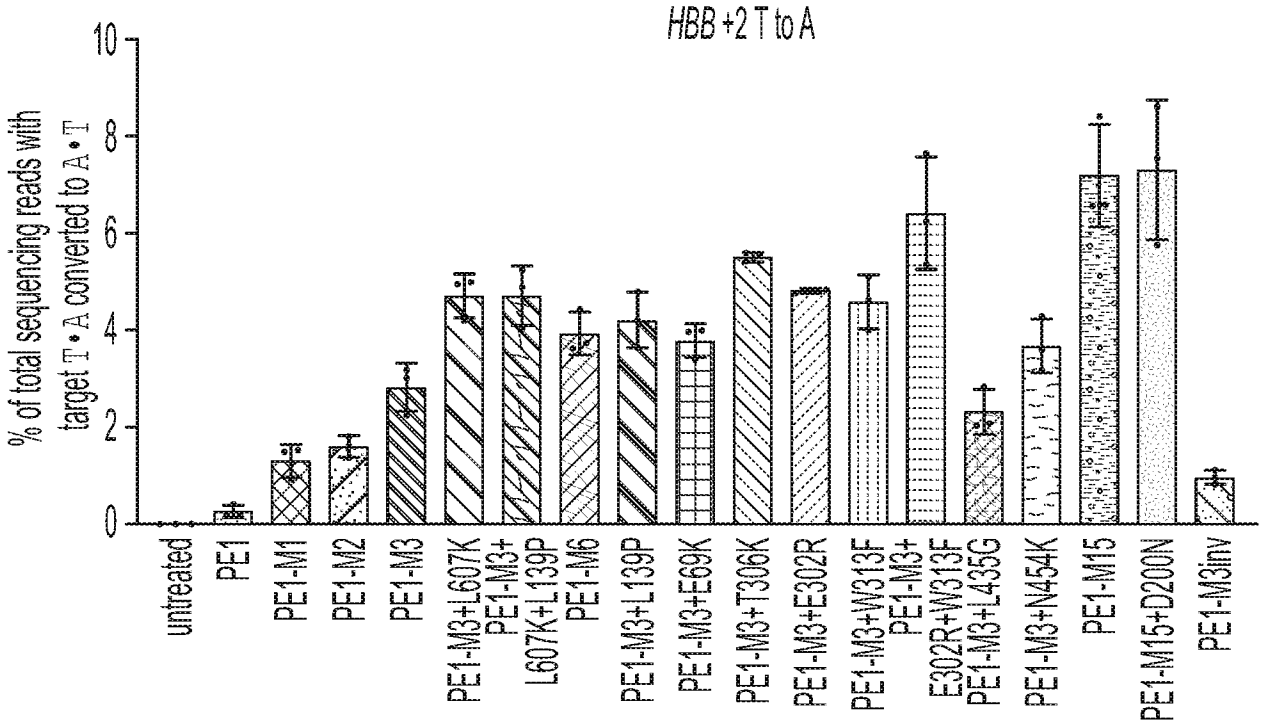


FIG. 47G

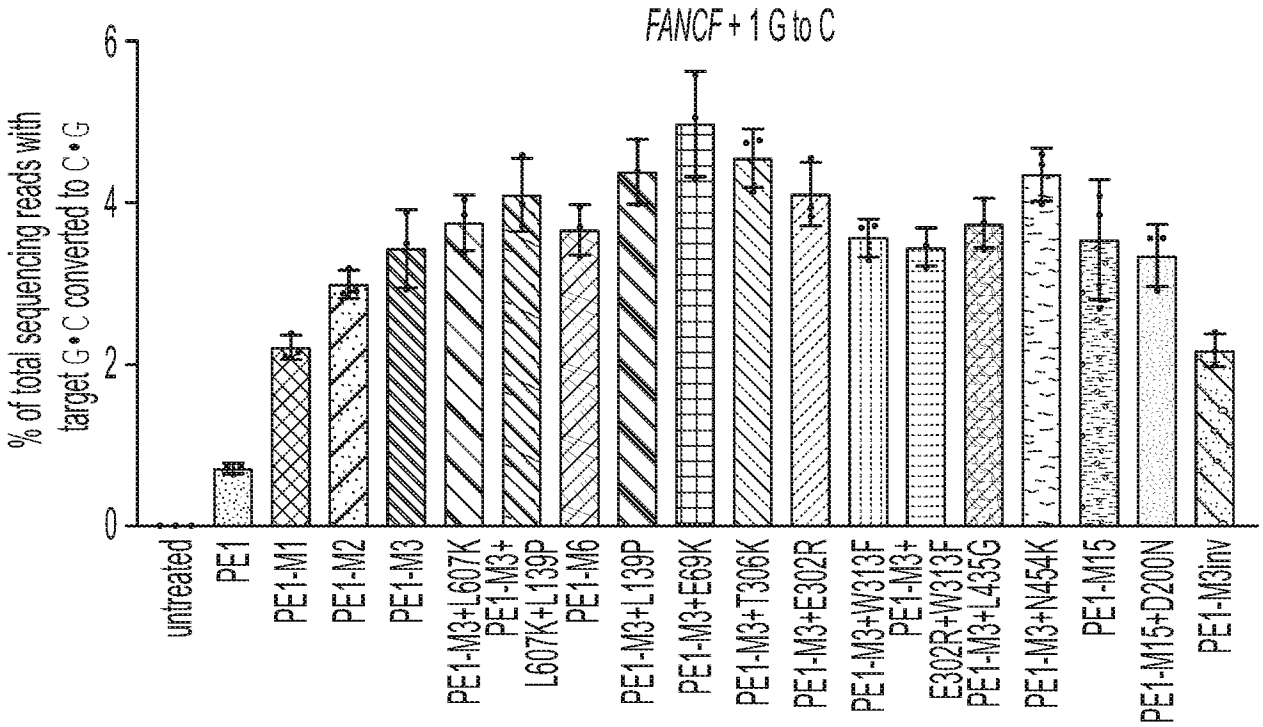


FIG. 47H

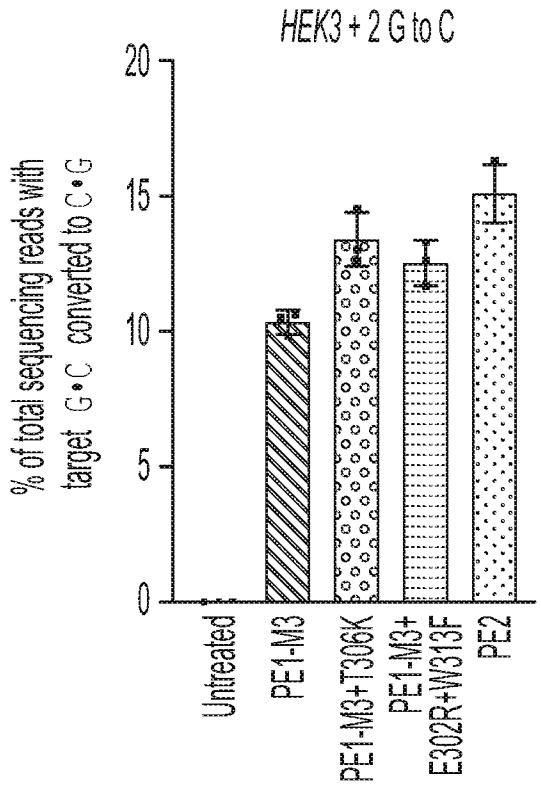


FIG. 47I

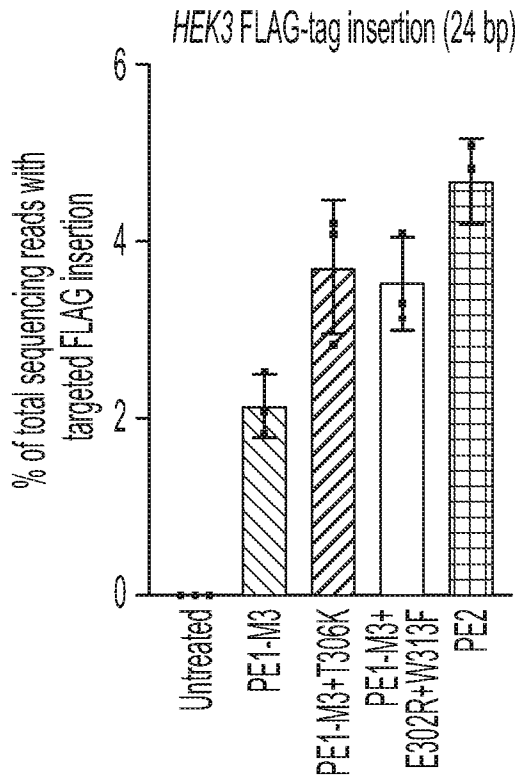


FIG. 47J

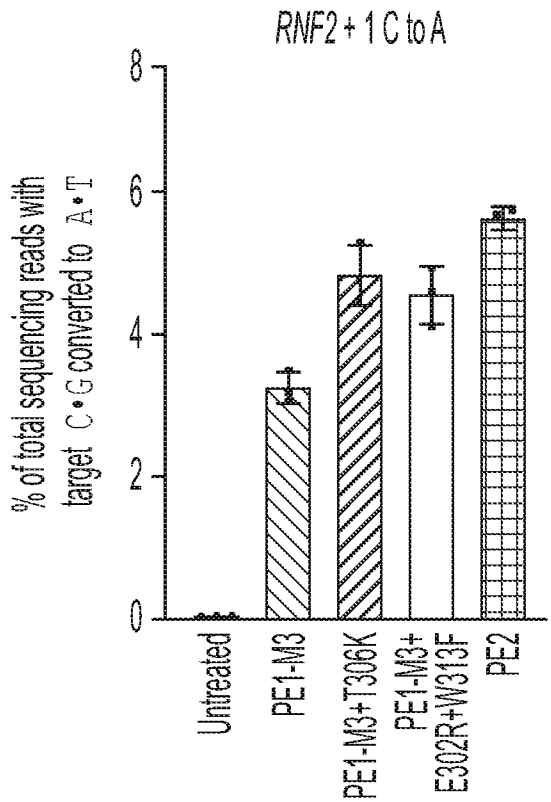


FIG. 47K

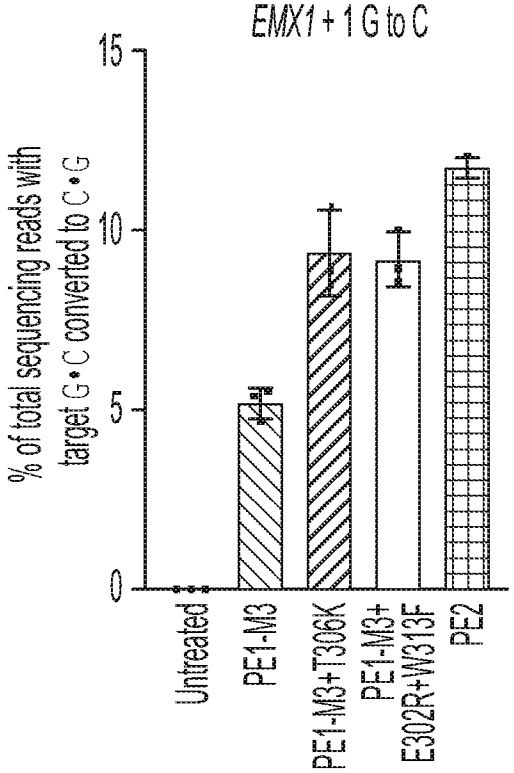


FIG. 47L

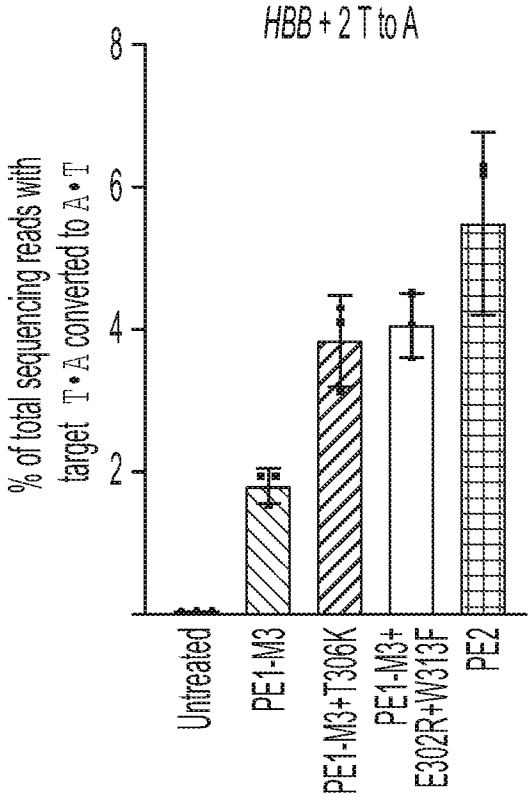


FIG. 47M

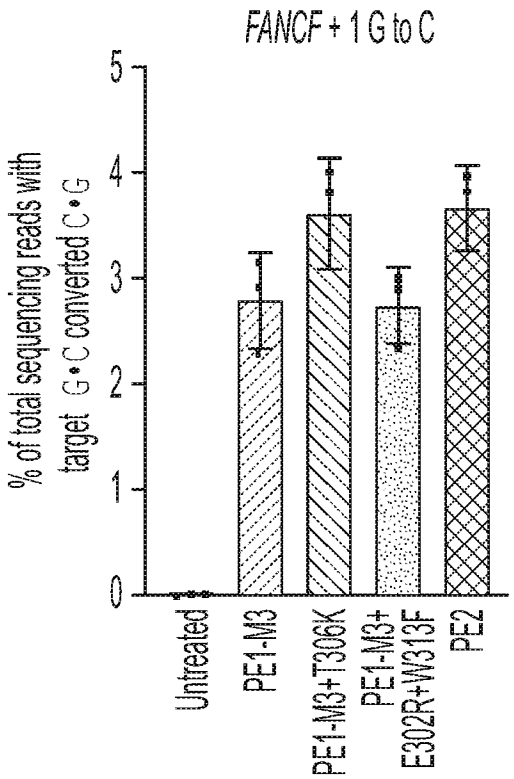


FIG. 47N

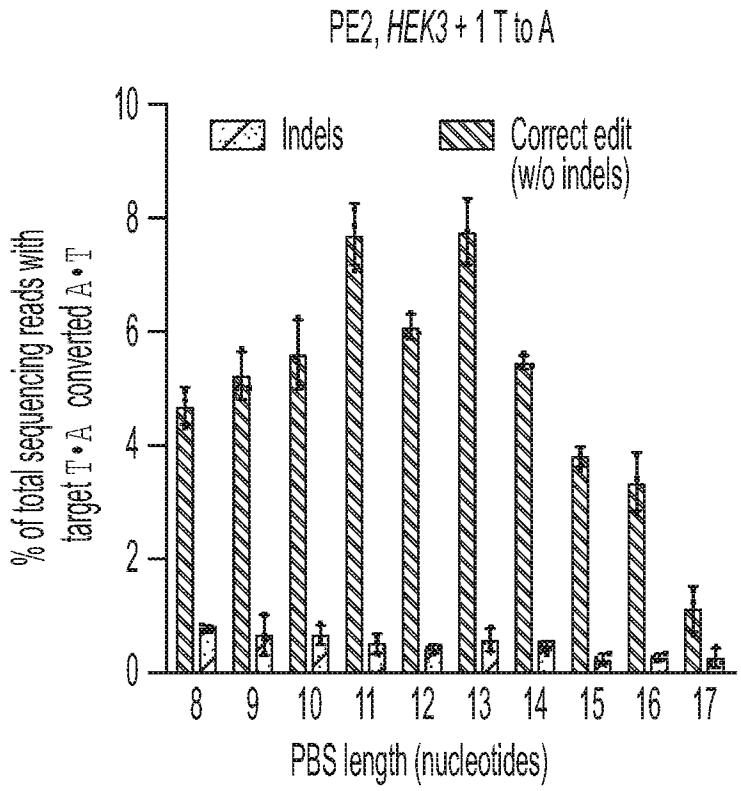


FIG. 47O

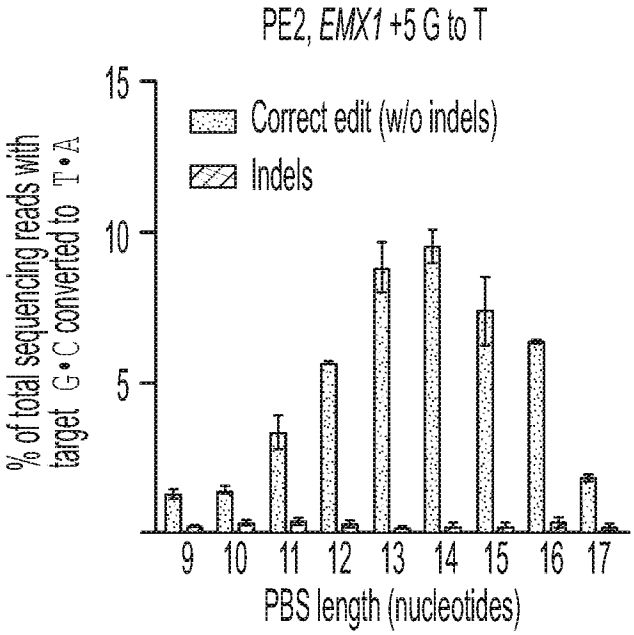


FIG. 47P

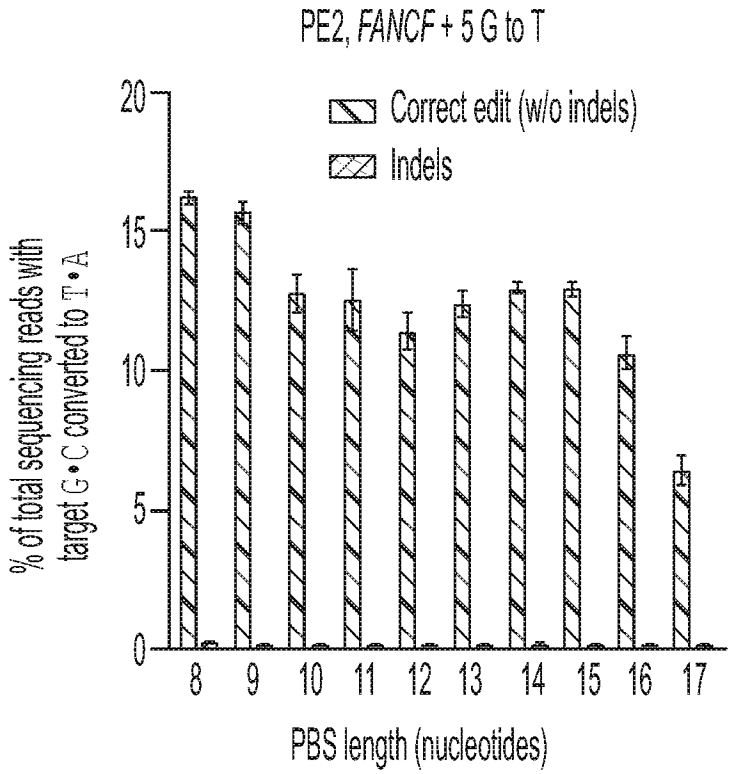


FIG. 47Q

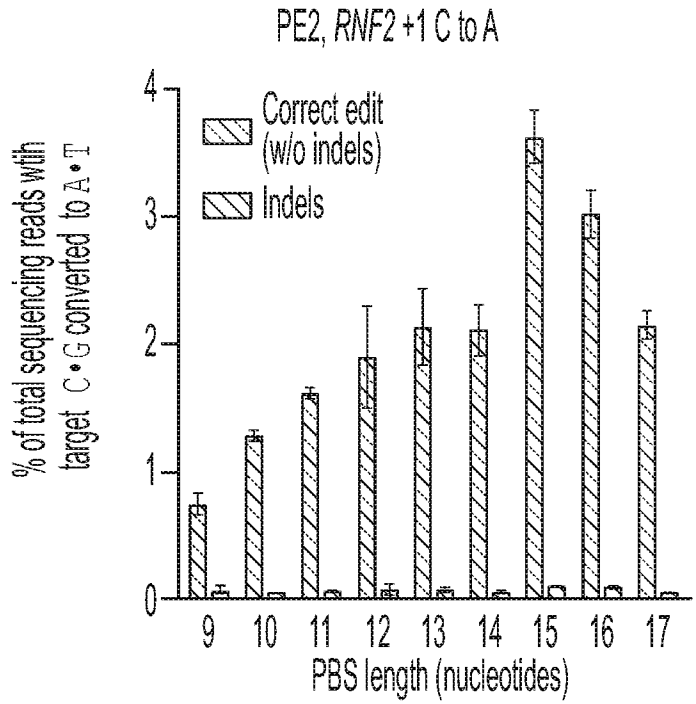


FIG. 47R

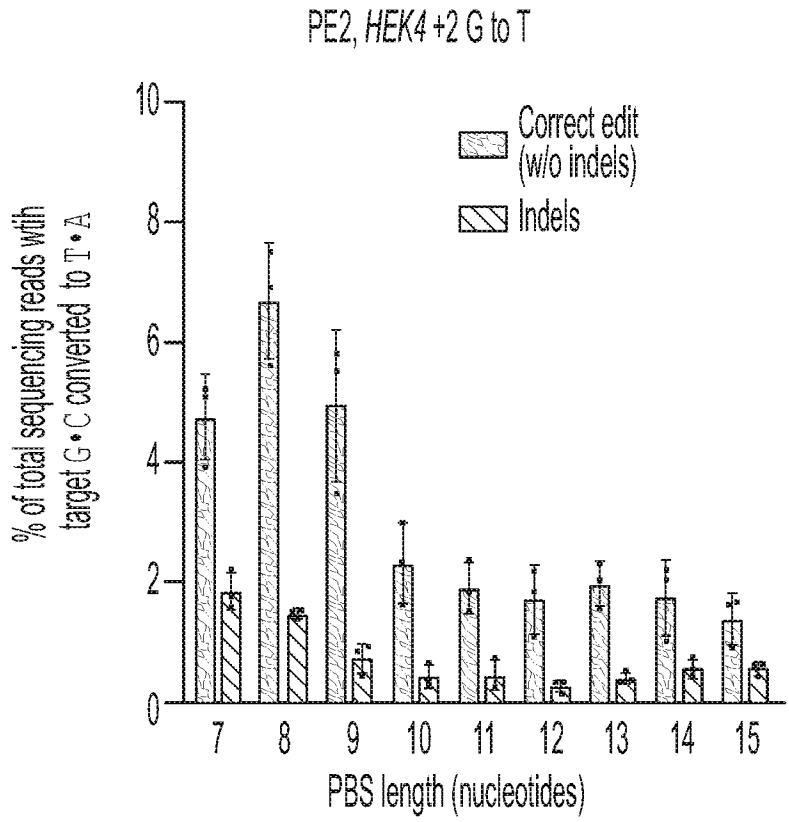


FIG. 47S

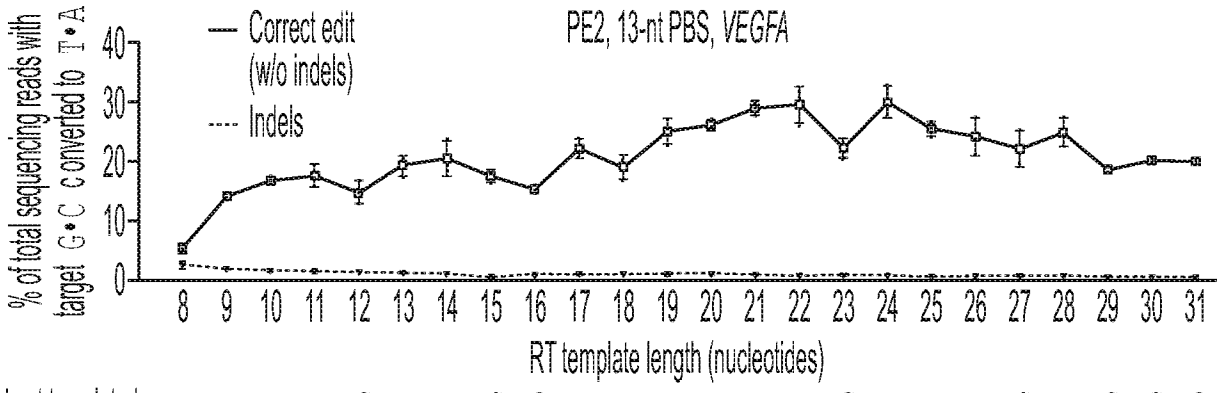


FIG. 48A

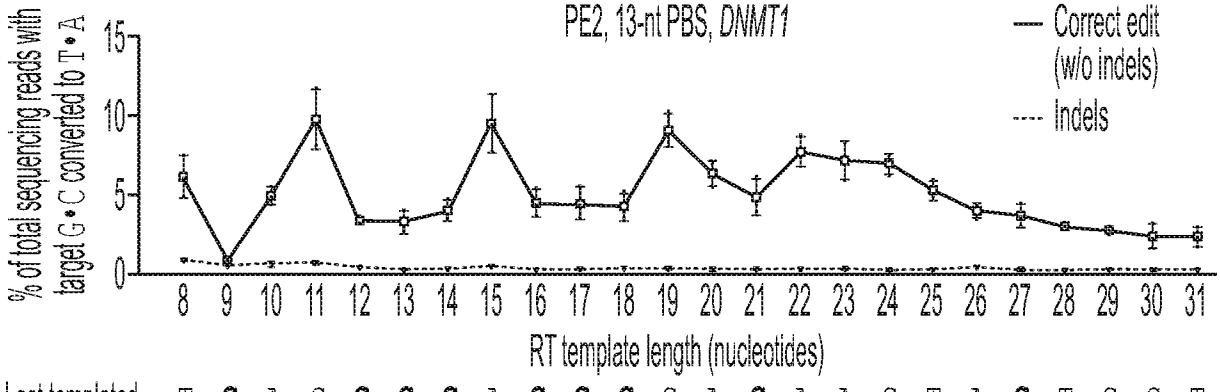


FIG. 48B

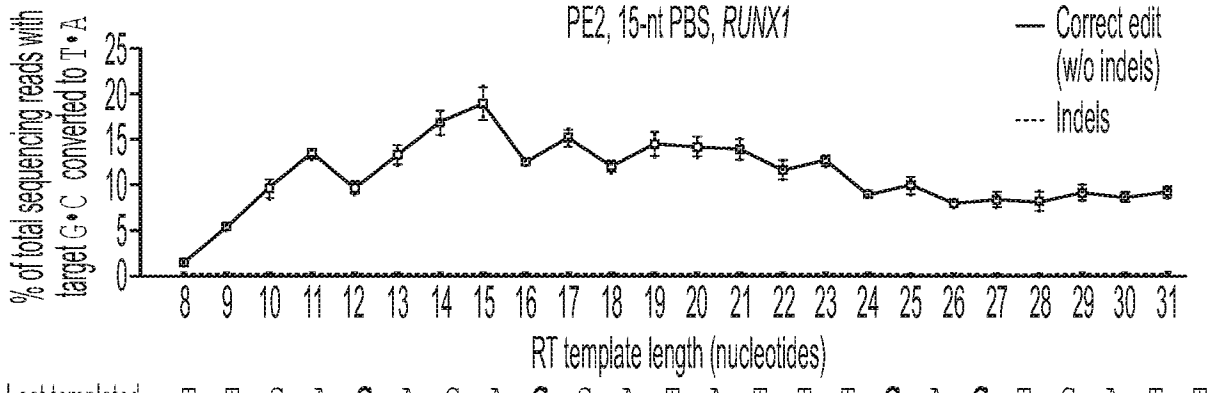


FIG. 48C

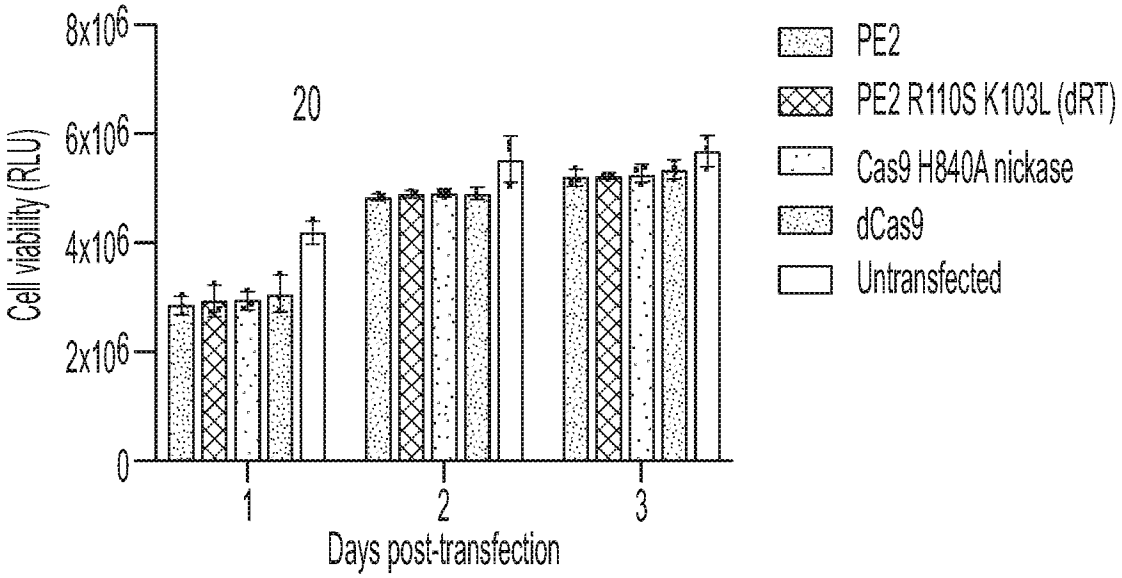


FIG. 49A

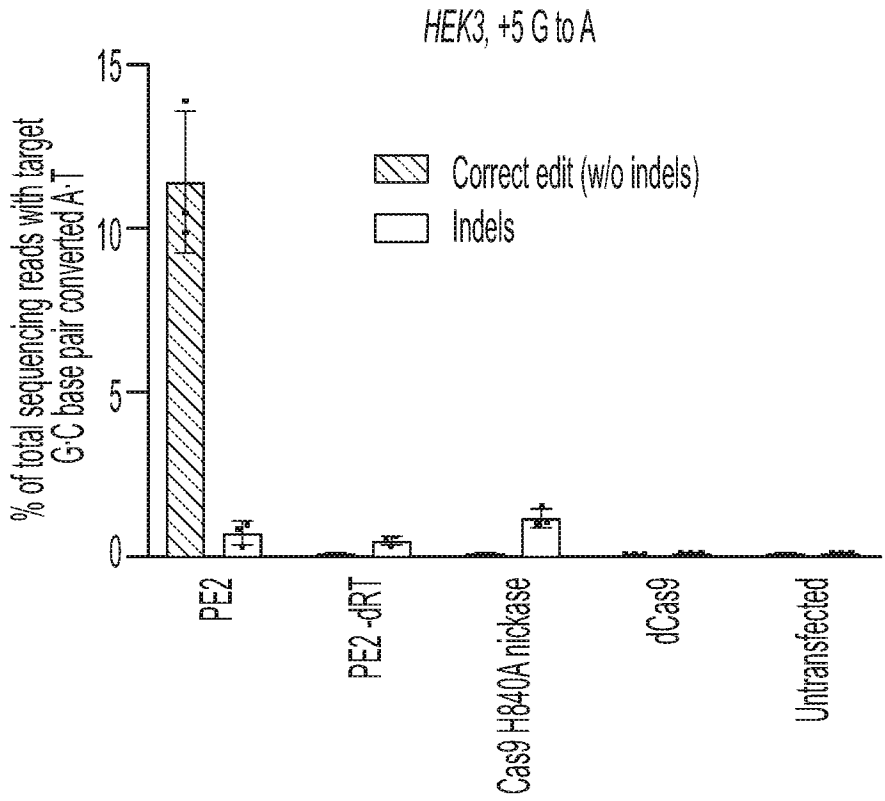


FIG. 49B

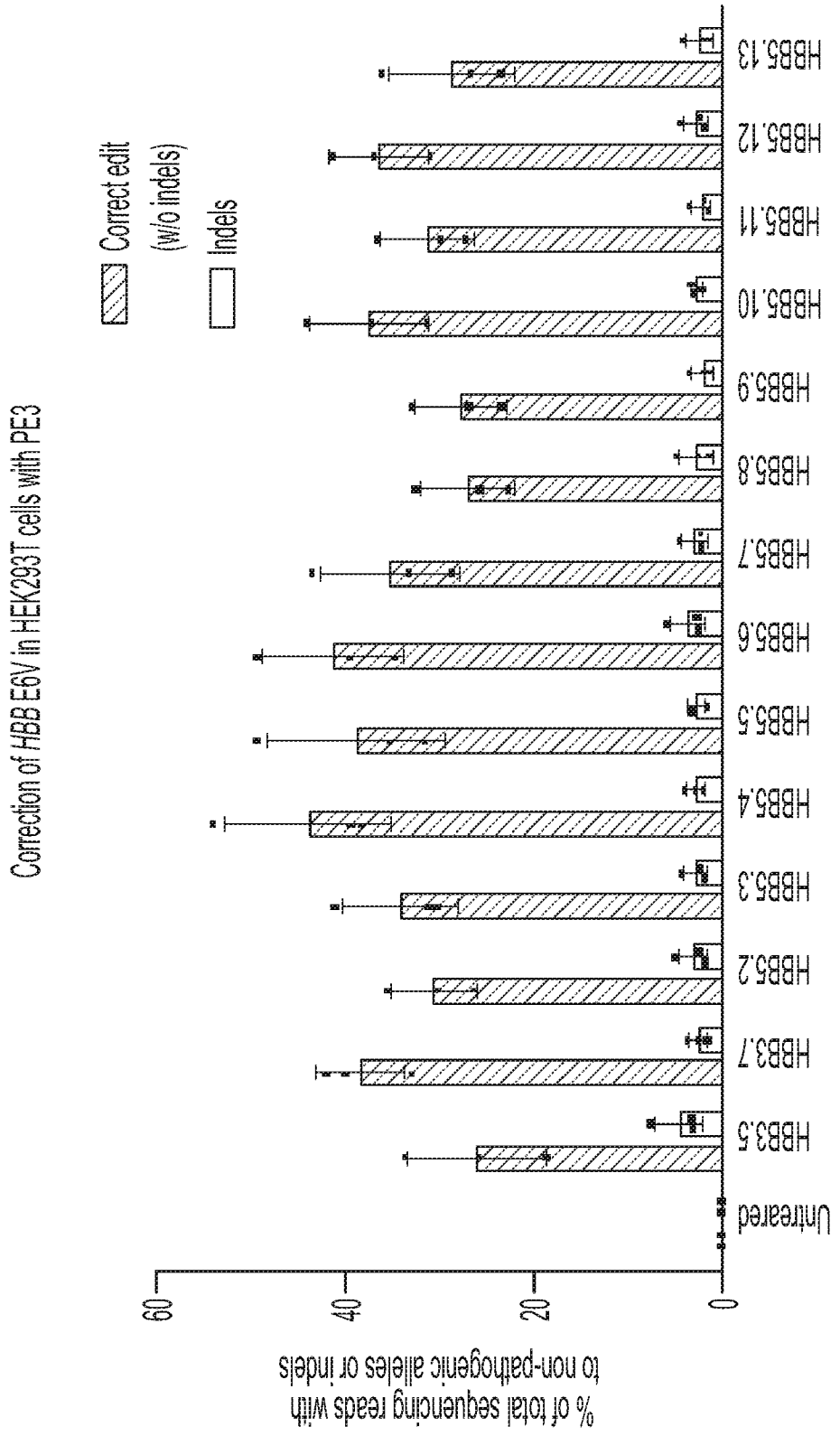

Correction of HBB E6V in HEK293T cells with PE3
Correct edit (w/o indels)
Indels
% of total sequencing reads with to non-pathogenic alleles or indels
0
20
40
60
Unlreared
HBB3.5
HBB3.7
HBB5.2
HBB5.3
HBB5.4
HBB5.5
HBB5.6
HBB5.7
HBB5.8
HBB5.9
HBB5.10
HBB5.11
HBB5.12
HBB5.13
pegRNA

FIG. 50A

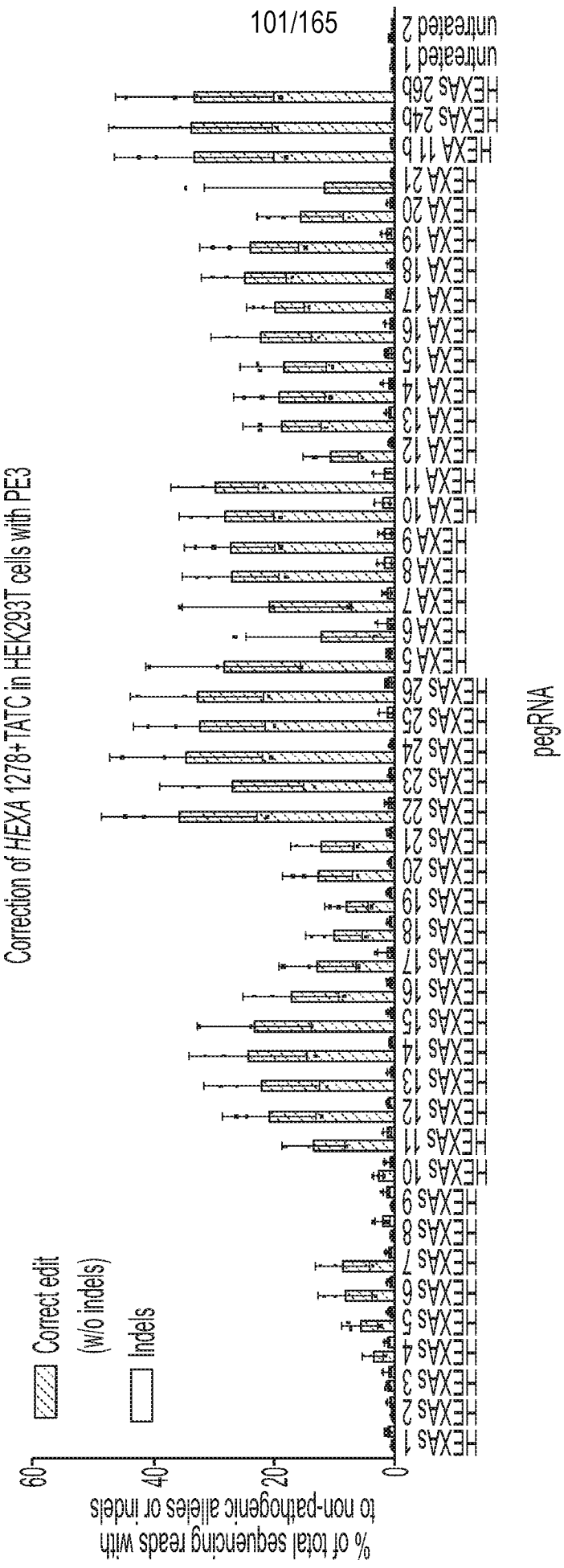

Correction of HEXA 1278+TATC in HEK293T cells with PE3
% of total sequencing reads with to non-pathogenic alleles or indels
0
20
40
60
Indels
Correct edit (w/o indels)
pegRNA
HEXAs 1
HEXAs 2
HEXAs 3
HEXAs 4
HEXAs 5
HEXAs 6
HEXAs 7
HEXAs 8
HEXAs 9
HEXAs 10
HEXAs 11
HEXAs 12
HEXAs 13
HEXAs 14
HEXAs 15
HEXAs 16
HEXAs 17
HEXAs 18
HEXAs 19
HEXAs 20
HEXAs 21
HEXAs 22
HEXAs 23
HEXAs 24
HEXAs 25
HEXAs 26
HEXA 5
HEXA 6
HEXA 7
HEXA 8
HEXA 9
HEXA 10
HEXA 11
HEXA 12
HEXA 13
HEXA 14
HEXA 15
HEXA 16
HEXA 17
HEXA 18
HEXA 19
HEXA 20
HEXA 21
HEXA 11 b
HEXAs 24b
HEXAs 26b
untreated 1
untreated 2

FIG. 50B

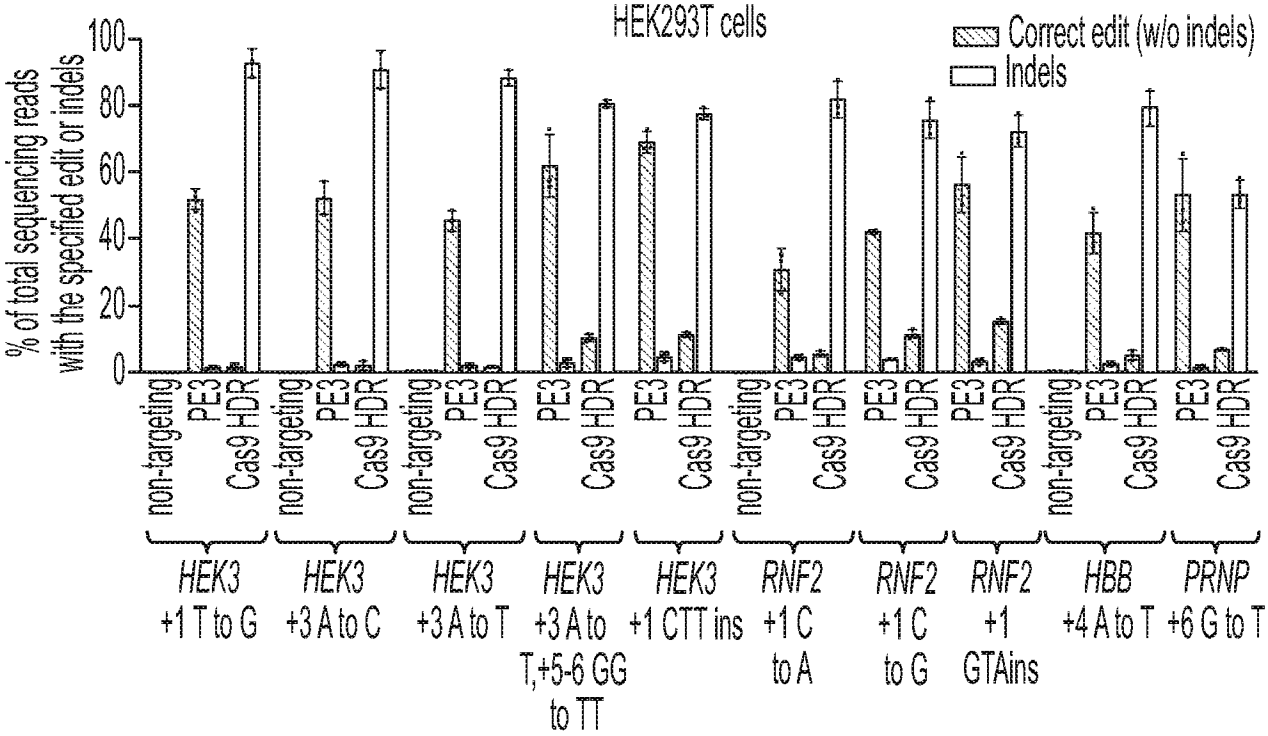


FIG. 51A

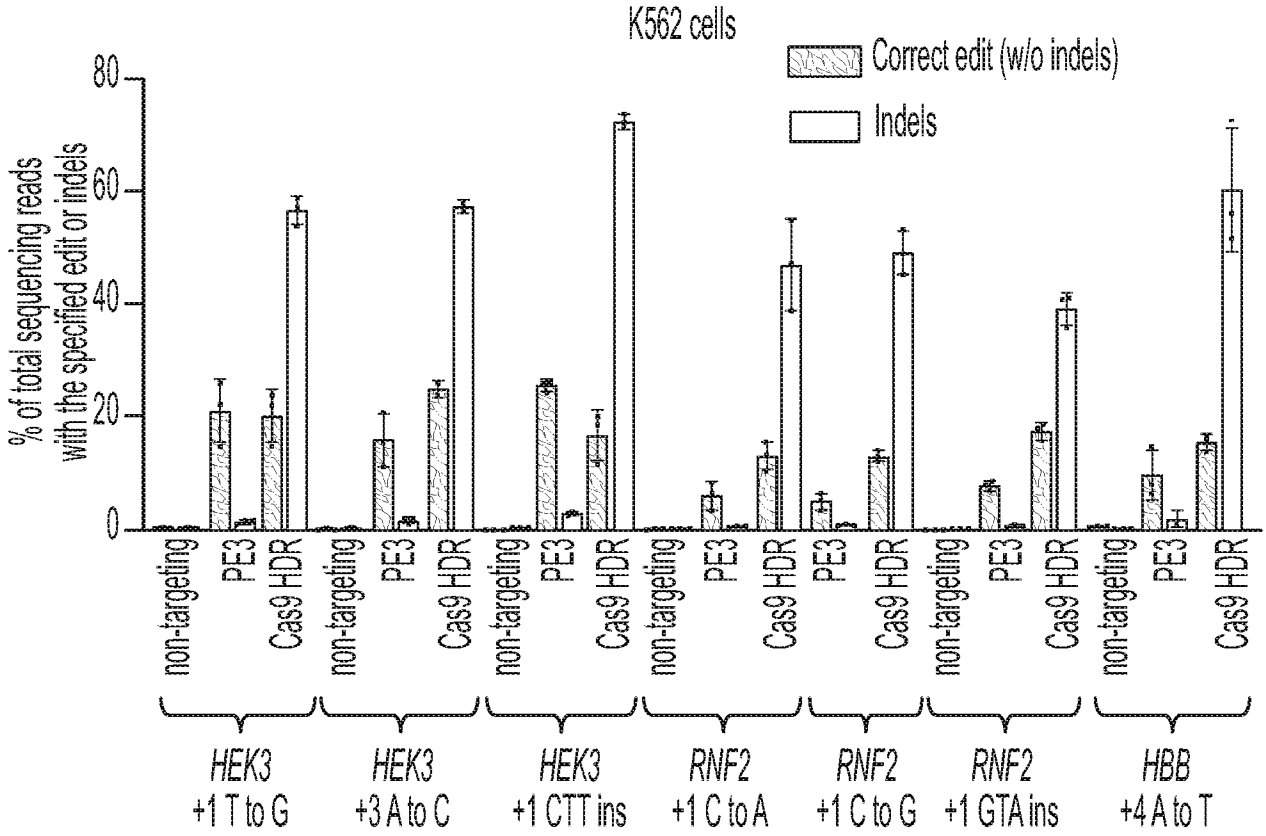


FIG. 51B

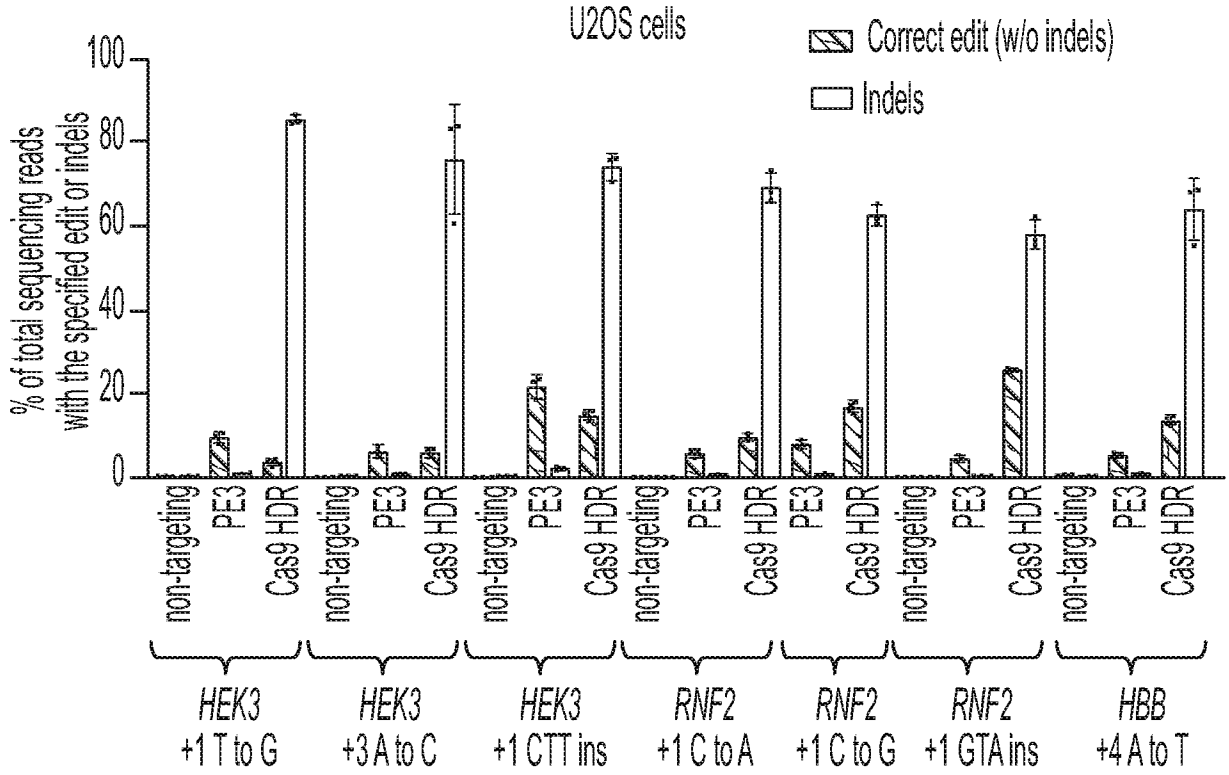


FIG. 51C

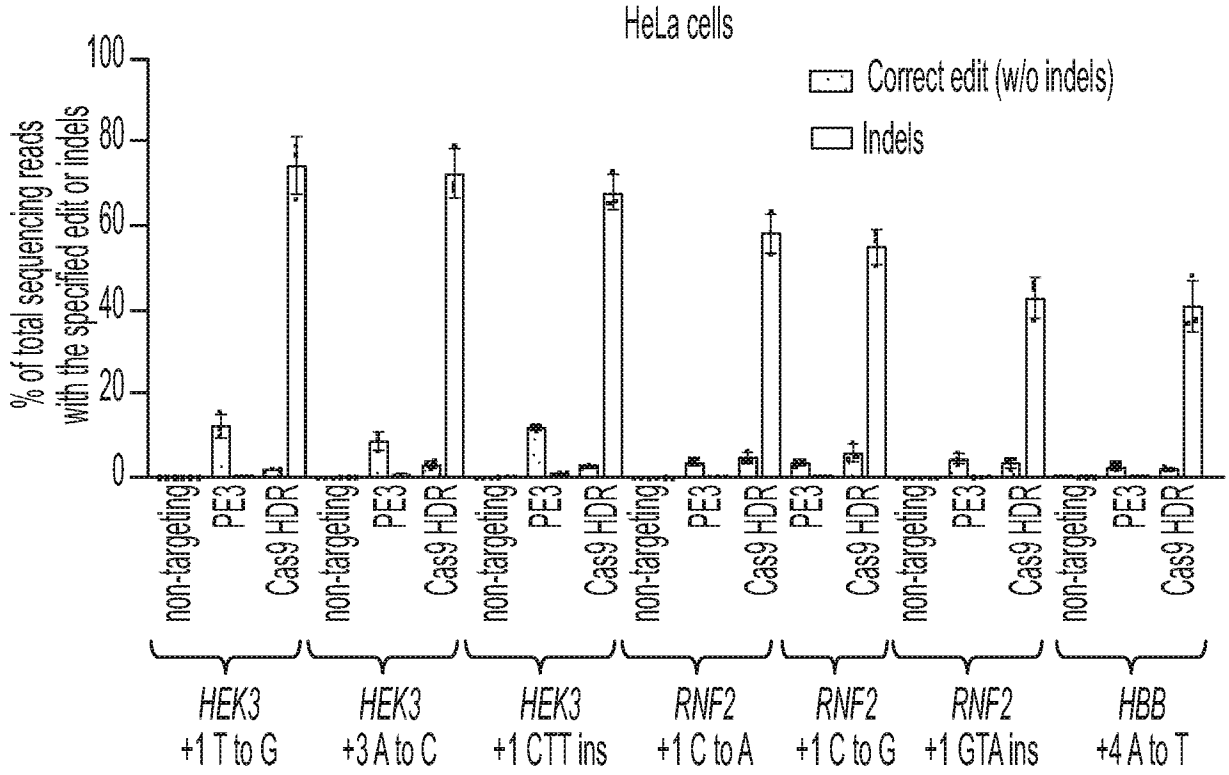


FIG. 51D

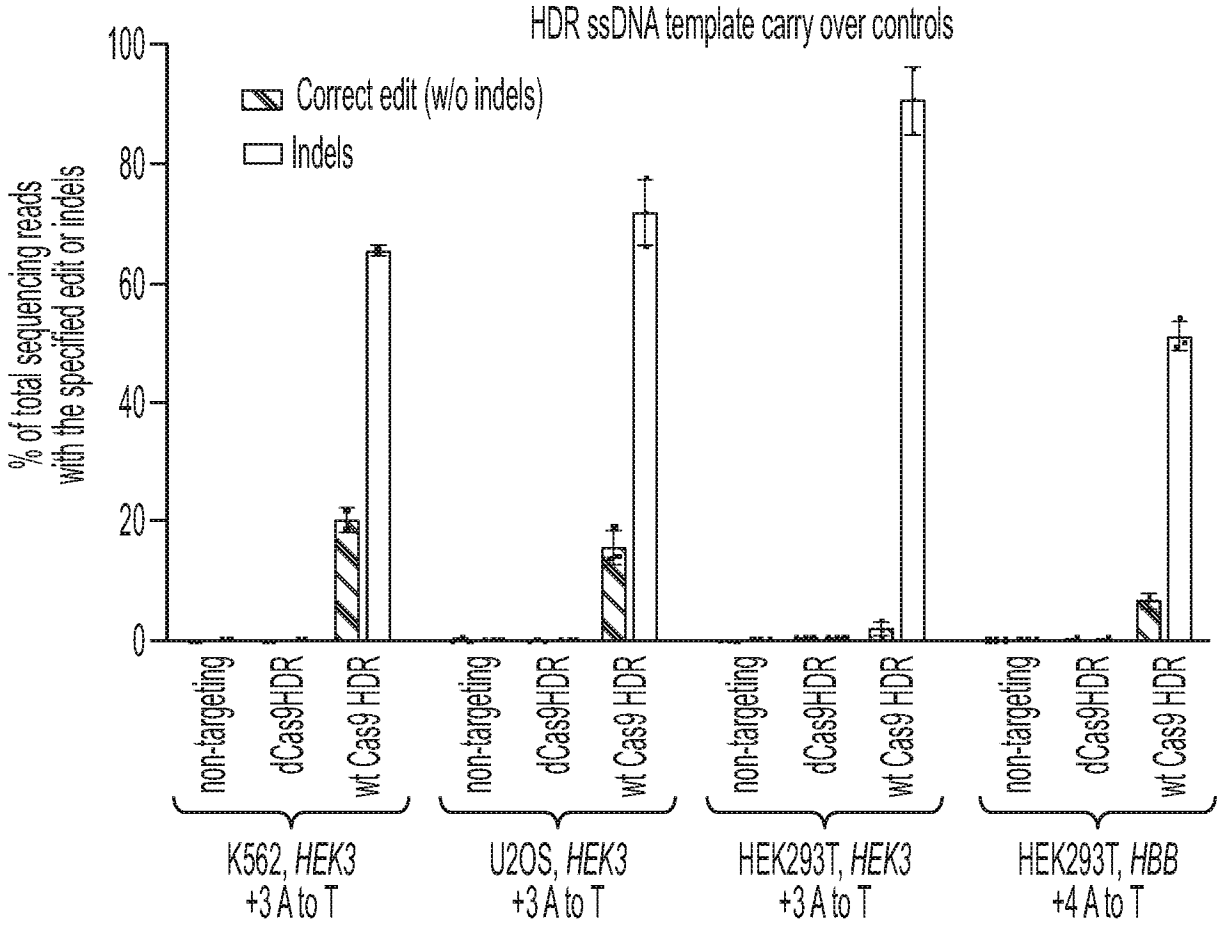

HDR ssDNA template carry over controls
Correct edit (w/o indels)
Indels
% of total sequencing reads with the specified edit or indels
100
80
60
40
20
0
non-targeting
dCas9HDR
wt Cas9 HDR
K562, HEK3 +3 A to T
U2OS, HEK3 +3 A to T
HEK293T, HEK3 +3 A to T
HEK293T, HBB +4 A to T

FIG. 51E

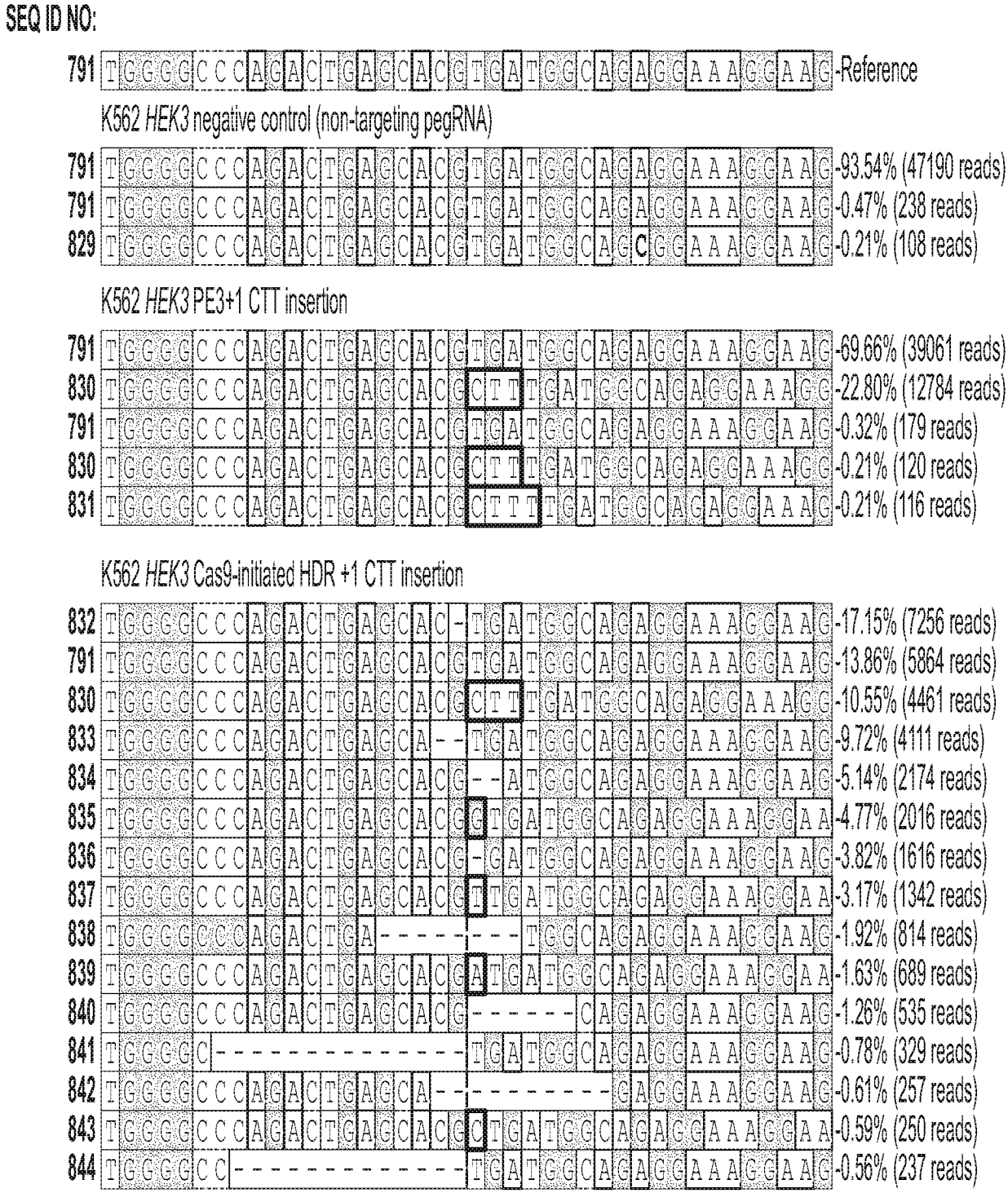

SEQ ID NO:
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -Reference
K562 HEK3 negative control (non-targeting pegRNA)
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -93.54% (47190 reads)
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -0.47% (238 reads)
829 TGGGGCCCAGACTGAGCACGTGATGGCAGCGGAAAGGAAG -0.21% (108 reads)
K562 HEK3 PE3+1 CTT insertion
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -69.66% (39061 reads)
830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG -22.80% (12784 reads)
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -0.32% (179 reads)
830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG -0.21% (120 reads)
831 TGGGGCCCAGACTGAGCACGCTTTTGATGGCAGAGGAAAG -0.21% (116 reads)
K562 HEK3 Cas9-initiated HDR +1 CTT insertion
832 TGGGGCCCAGACTGAGCAC-TGATGGCAGAGGAAAGGAAG -17.15% (7256 reads)
791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -13.86% (5864 reads)
830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG -10.55% (4461 reads)
833 TGGGGCCCAGACTGAGCA--TGATGGCAGAGGAAAGGAAG -9.72% (4111 reads)
834 TGGGGCCCAGACTGAGCACG--ATGGCAGAGGAAAGGAAG -5.14% (2174 reads)
835 TGGGGCCCAGACTGAGCACGGTGATGGCAGAGGAAAGGAA -4.77% (2016 reads)
836 TGGGGCCCAGACTGAGCACG-GATGGCAGAGGAAAGGAAG -3.82% (1616 reads)
837 TGGGGCCCAGACTGAGCACGTTGATGGCAGAGGAAAGGAA -3.17% (1342 reads)
838 TGGGGCCCAGACTGA-----TGGCAGAGGAAAGGAAG -1.92% (814 reads)
839 TGGGGCCCAGACTGAGCACGATGATGGCAGAGGAAAGGAA -1.63% (689 reads)
840 TGGGGCCCAGACTGAGCACG------CAGAGGAAAGGAAG -1.26% (535 reads)
841 TGGGGC----------TGATGGCAGAGGAAAGGAAG -0.78% (329 reads)
842 TGGGGCCCAGACTGAGCA---------GAGGAAAGGAAG -0.61% (257 reads)
843 TGGGGCCCAGACTGAGCACGCTGATGGCAGAGGAAAGGAA -0.59% (250 reads)
844 TGGGGCC----------TGATGGCAGAGGAAAGGAAG -0.56% (237 reads)

FIG. 51F

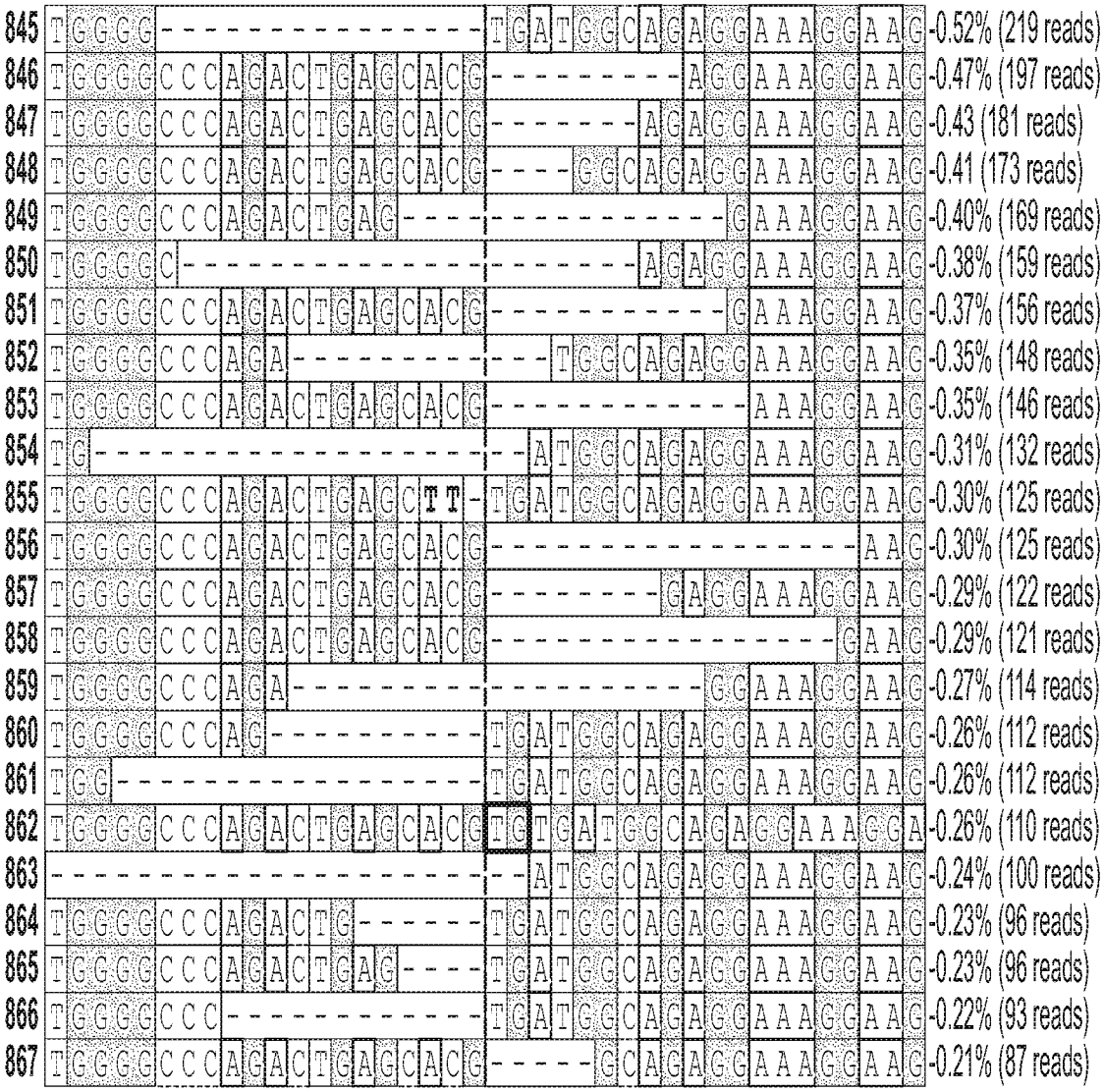


FIG. 51F
CONTINUED

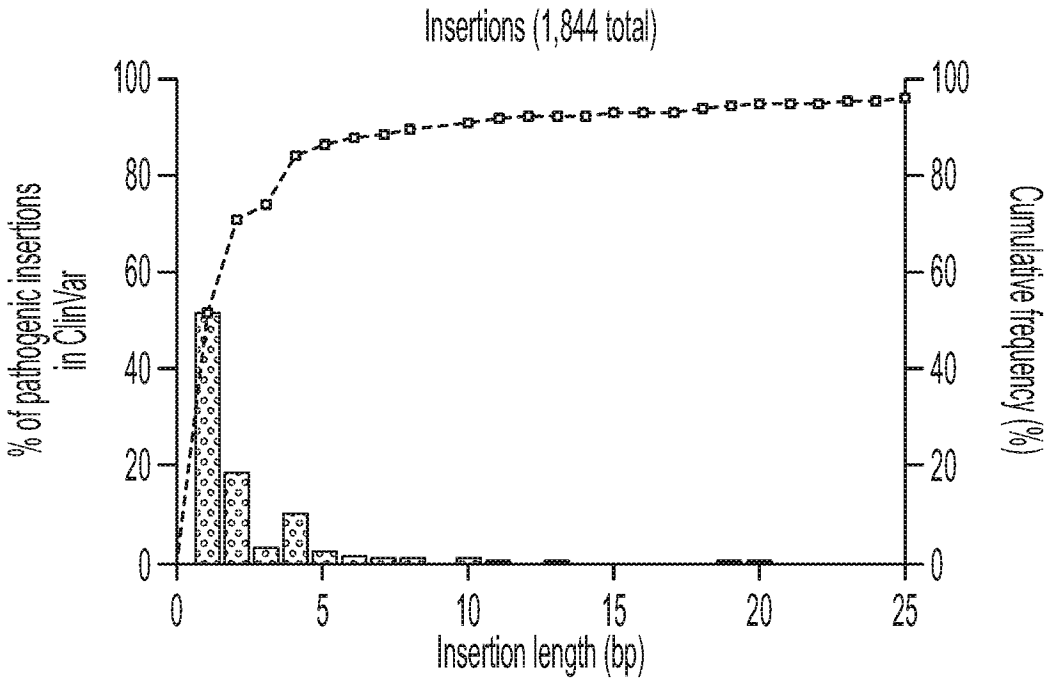


FIG. 52A

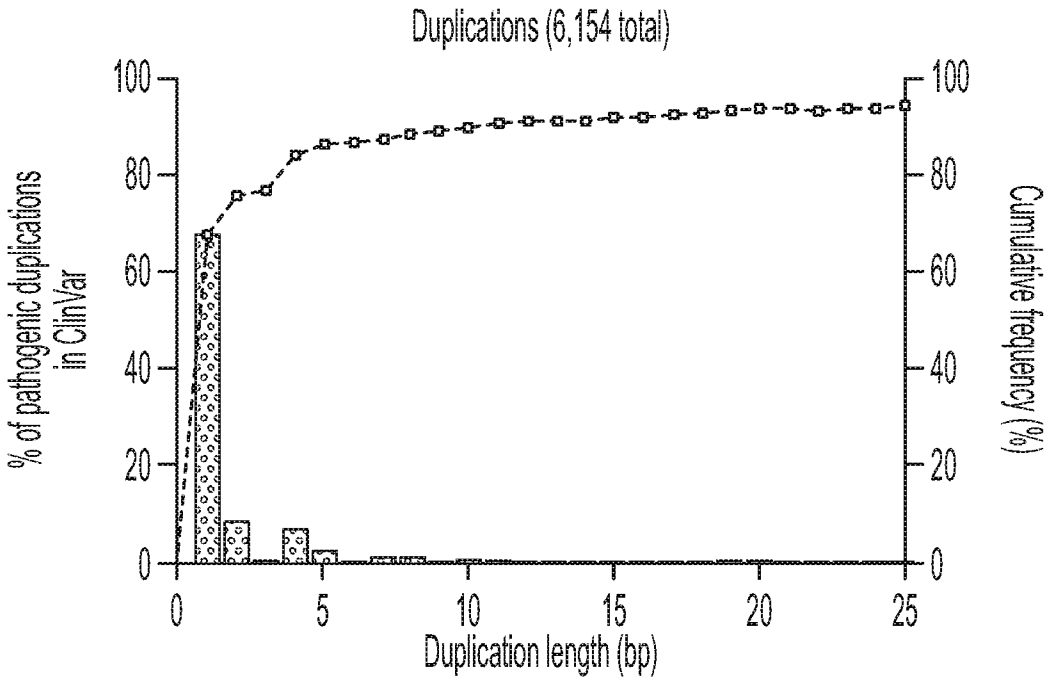


FIG. 52B

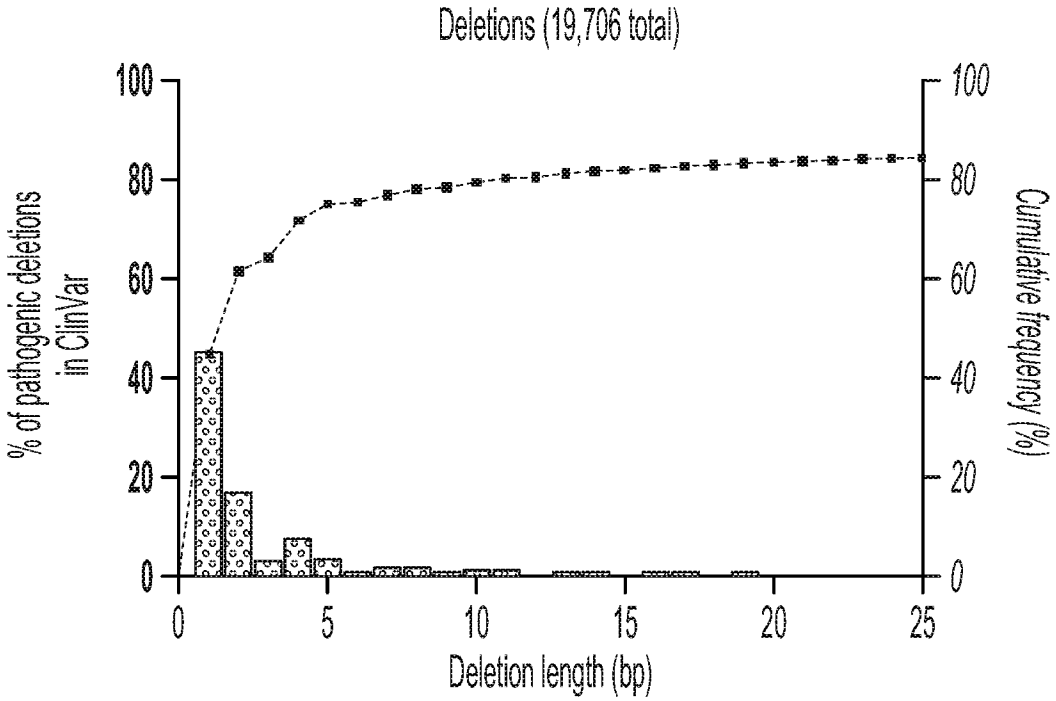


FIG. 52C

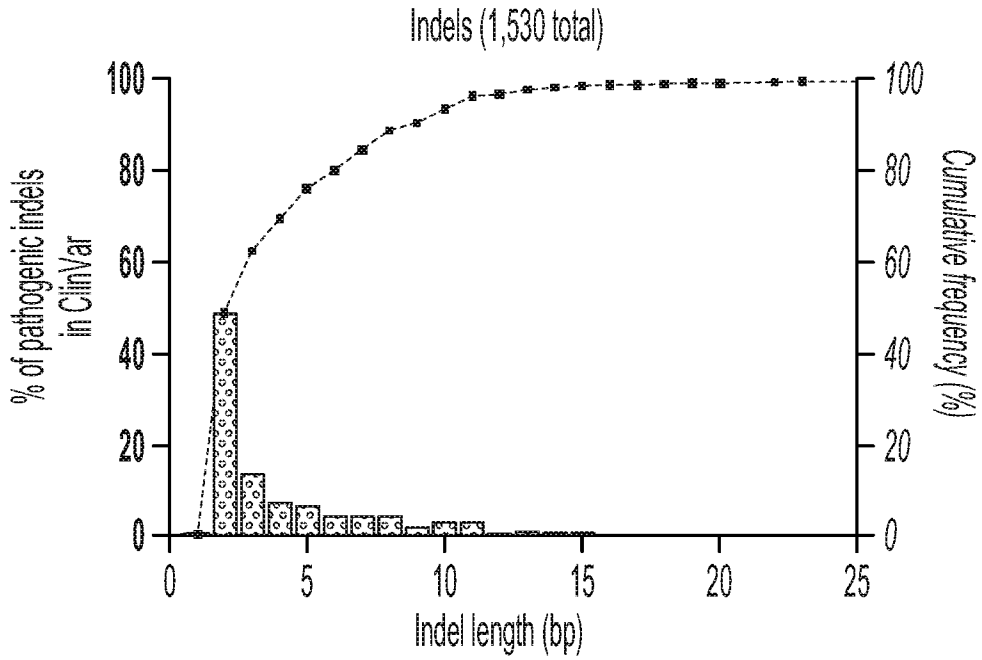


FIG. 52D

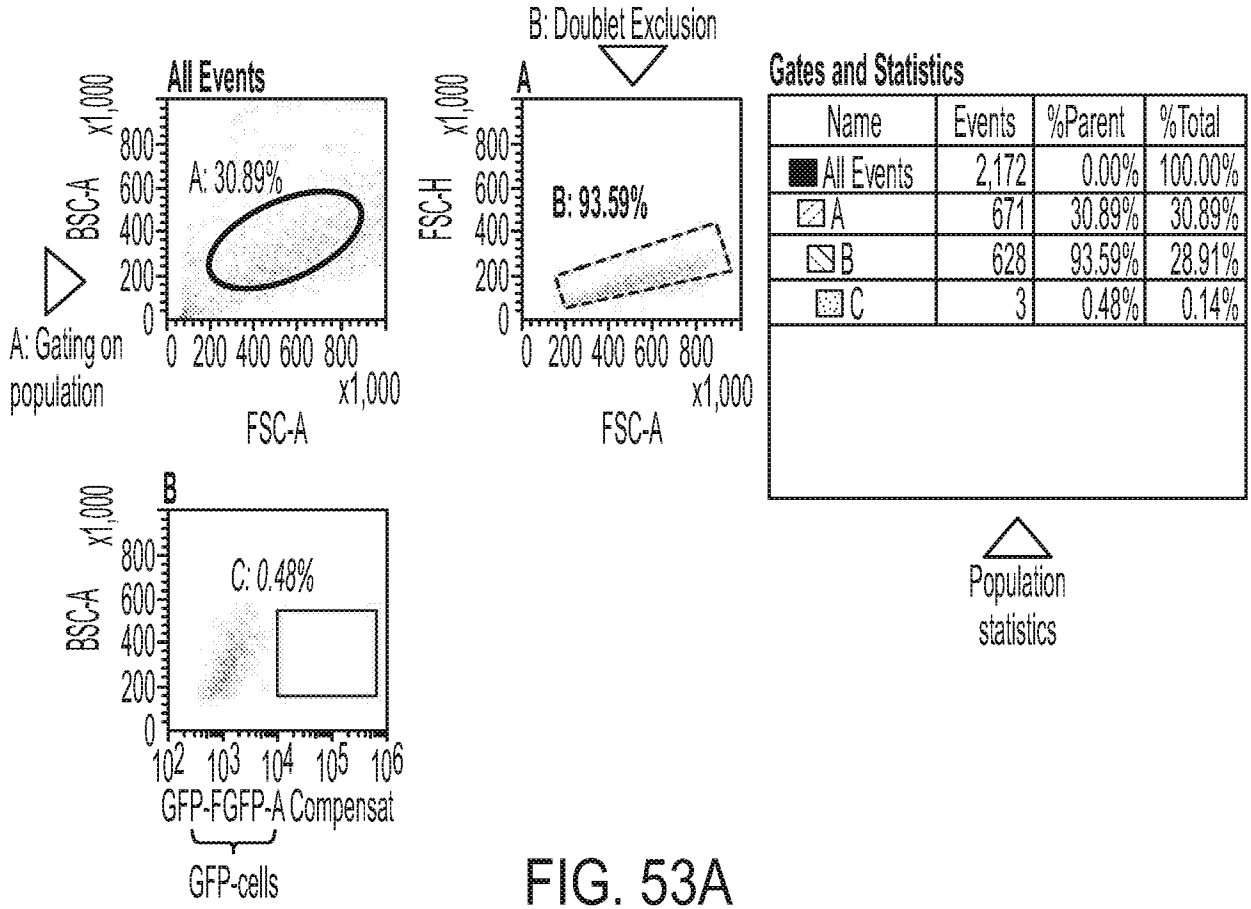


FIG. 53A

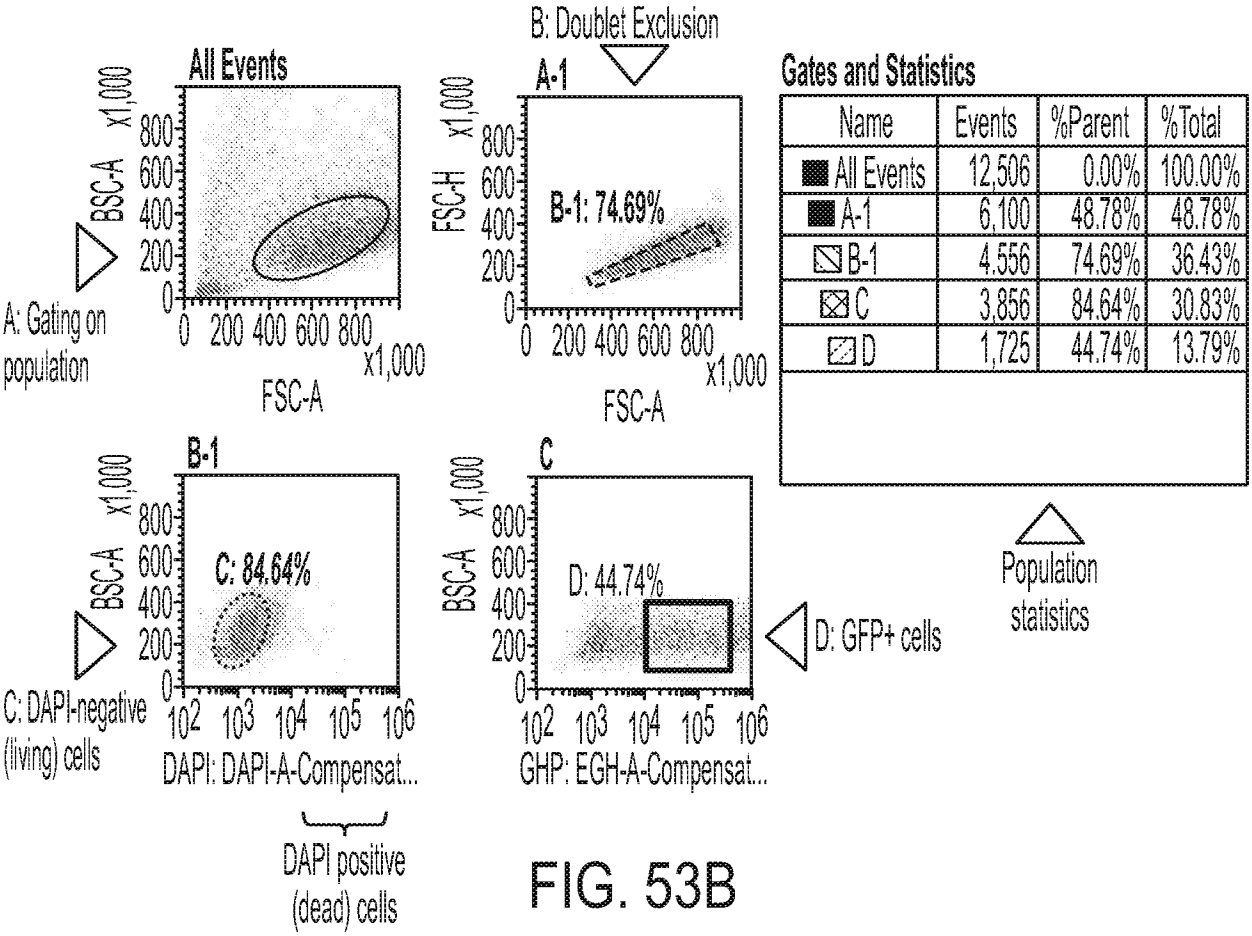


FIG. 53B

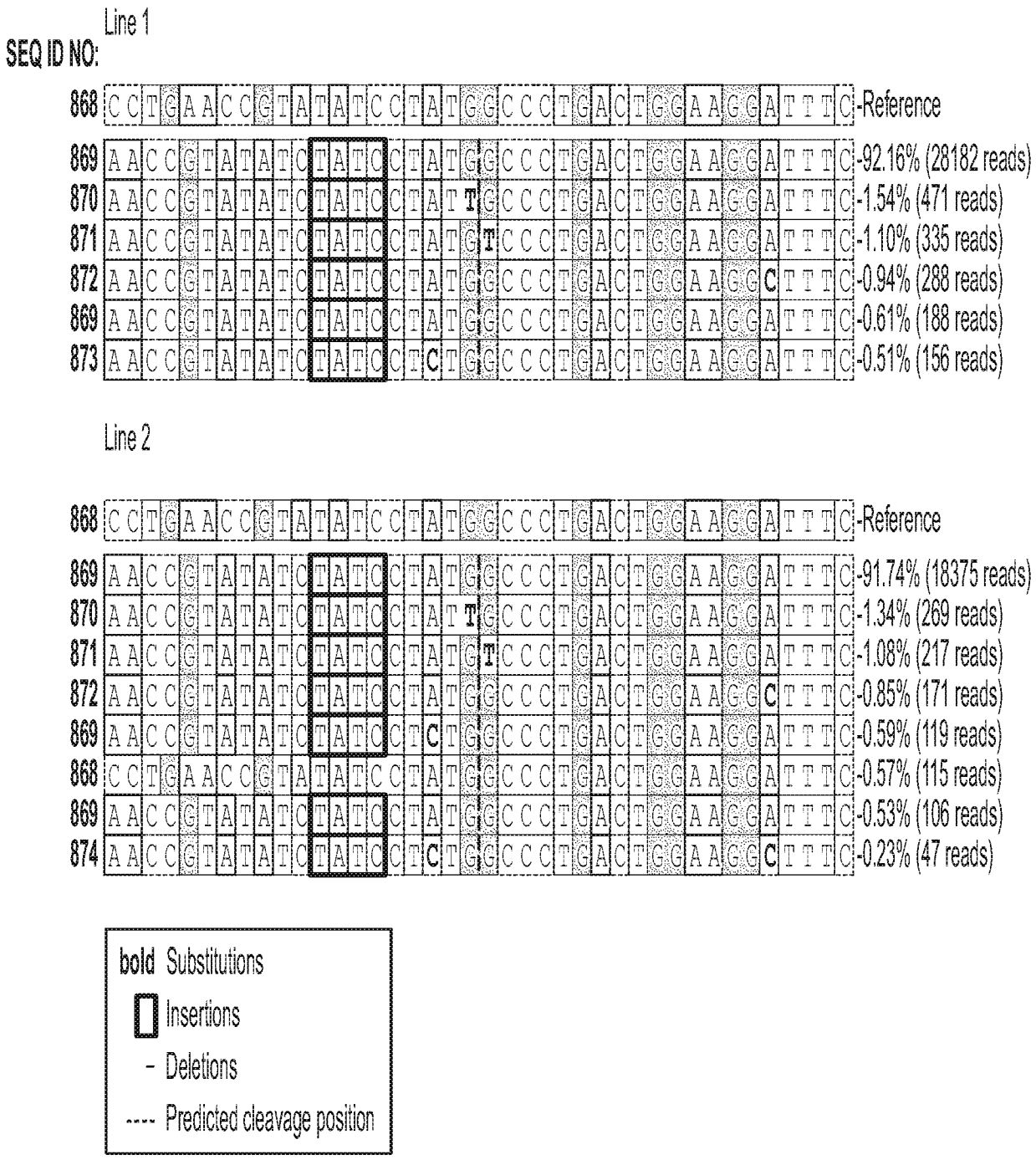

Line 1
SEQ ID NO:
868 C C T G A A C C G T A T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -Reference
869 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -92.16% (28182 reads)
870 A A C C G T A T A T C T A T C C T A T T G C C C T G A C T G G A A G G A T T T C -1.54% (471 reads)
871 A A C C G T A T A T C T A T C C T A T G T C C C T G A C T G G A A G G A T T T C -1.10% (335 reads)
872 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G C T T T C -0.94% (288 reads)
869 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -0.61% (188 reads)
873 A A C C G T A T A T C T A T C C T C T G G C C C T G A C T G G A A G G A T T T C -0.51% (156 reads)
Line 2
868 C C T G A A C C G T A T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -Reference
869 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -91.74% (18375 reads)
870 A A C C G T A T A T C T A T C C T A T T G C C C T G A C T G G A A G G A T T T C -1.34% (269 reads)
871 A A C C G T A T A T C T A T C C T A T G T C C C T G A C T G G A A G G A T T T C -1.08% (217 reads)
872 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G C T T T C -0.85% (171 reads)
869 A A C C G T A T A T C T A T C C T C T G G C C C T G A C T G G A A G G A T T T C -0.59% (119 reads)
868 C C T G A A C C G T A T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -0.57% (115 reads)
869 A A C C G T A T A T C T A T C C T A T G G C C C T G A C T G G A A G G A T T T C -0.53% (106 reads)
874 A A C C G T A T A T C T A T C C T C T G G C C C T G A C T G G A A G G C T T T C -0.23% (47 reads)
bold Substitutions
Insertions
- Deletions
---- Predicted cleavage position

FIG. 53C

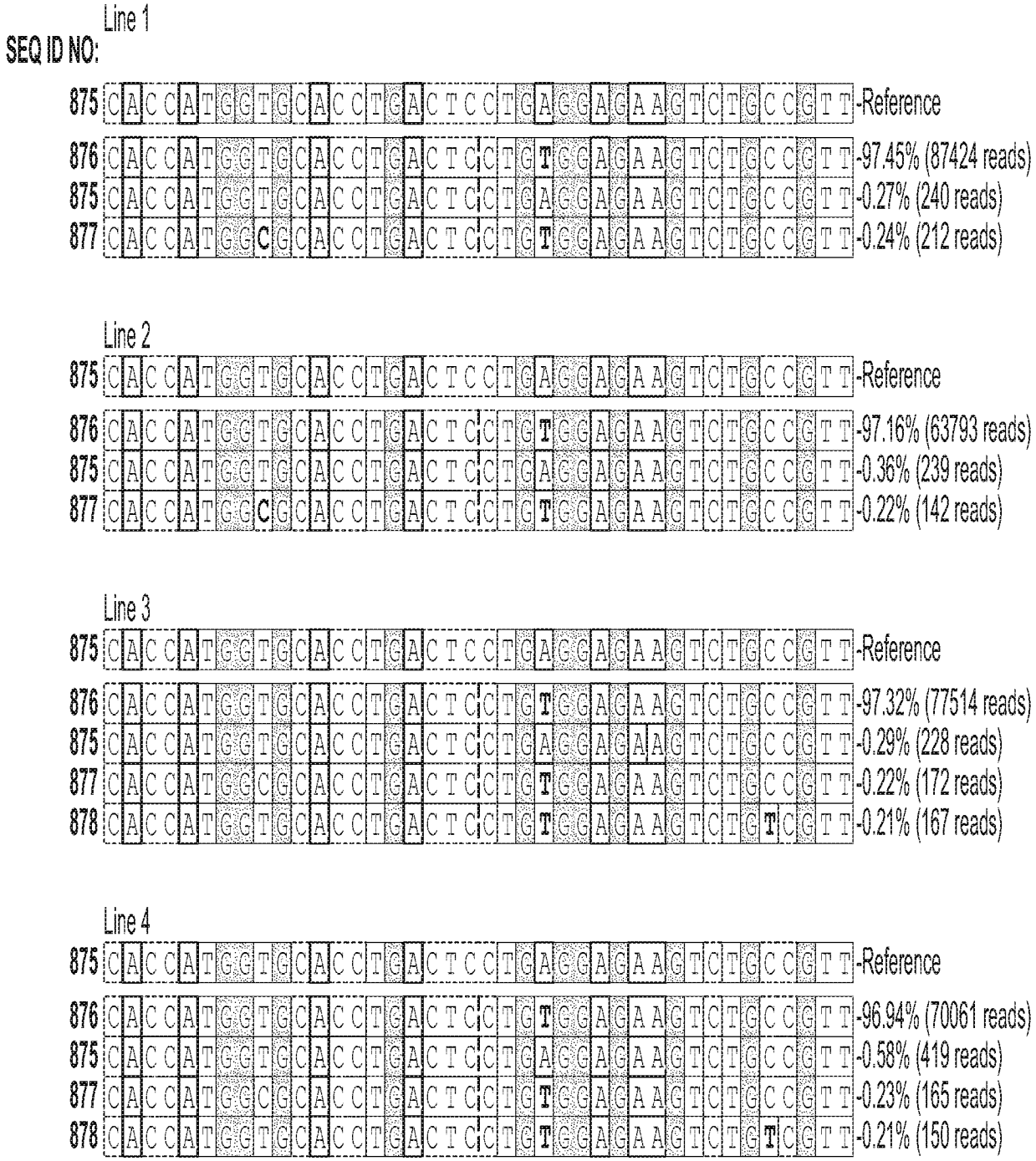

Line 1
SEQ ID NO:
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -97.45% (87424 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -0.27% (240 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.24% (212 reads)
Line 2
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -97.16% (63793 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -0.36% (239 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.22% (142 reads)
Line 3
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -97.32% (77514 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAATCTGCCGTT -0.29% (228 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.22% (172 reads)
878 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGTCGTT -0.21% (167 reads)
Line 4
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -96.94% (70061 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -0.58% (419 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.23% (165 reads)
878 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGTCGTT -0.21% (150 reads)

FIG. 53D

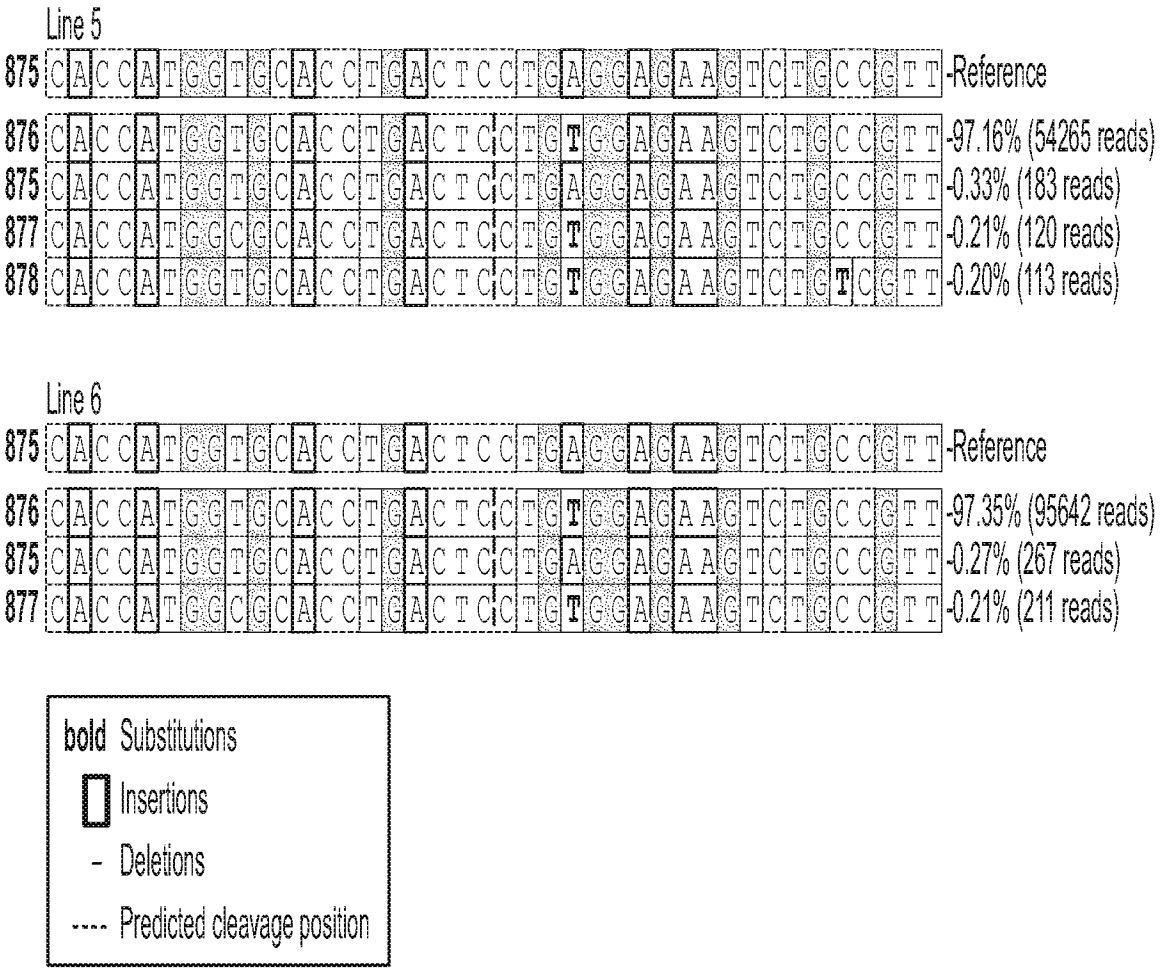
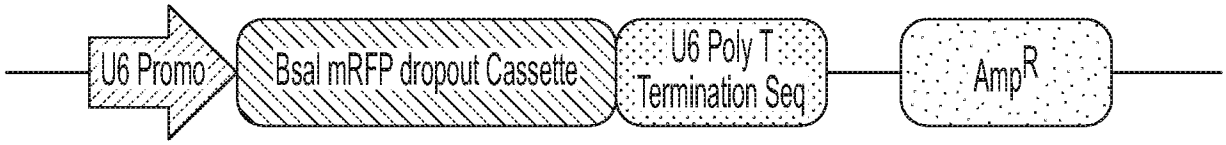


FIG. 53D
CONTINUED

Protocol to simultaneously clone a spacer sequence (Target Site) and pegRNA 3' extension containing a PBS and RT template into a human U6 promotor pegRNA expression vector:

[Component 1] Undigested pU6-pegRNA-GG-Vector Schematic:



Plasmid	Description	Features	Cut with
pU6-pegRNA-GG-Vector	Human pegRNA U6 expression vector	U6_BsaI-mRFP-cassette_U6-Term	BsaI-HFV2

[Component 2] Ordering oligonucleotides to generate the spacer (target) sequence for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector:

SEQ ID NO: 879 5′ - CACCNNNNNNNNNNNNNNNNNNNGTTTT - 3′

SEQ ID NO: 880 3′ - NNNNNNNNNNNNNNNNNNNCAAAATCTC - 5′

(The N20 mock protospacer sequence shown can be of variable length, as needed)

[Component 3] Ordering oligonucleotides to generate the SpCas9 sgRNA scaffold for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector

{**Phos**} 5′ - AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG - 3′ SEQ ID NO: 881

SEQ ID NO: 882 3′ - GATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCACG - 5′ {**Phos**}

(Note that these oligos **must be 5′ phosphorylated** to permit pegRNA vector assembly)

[Component 4] Ordering oligonucleotides to generate the pegRNA 3' extension containing a PBS and RT template sequence for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector:

SEQ ID NO: 883 5′ - GTGCNNNNNNNNNNNNNNNNNNNNNNNNNNNN - 3′

SEQ ID NO: 884 3′ - NNNNNNNNNNNNNNNNNNNNNNNNNNNNAAA - 5′

(The N30 mock reverse transcriptase template sequence shown can be of variable length, as needed)

Schematic of final assembled U6 pegRNA expression vector from components 1, 2, 3, and 4:

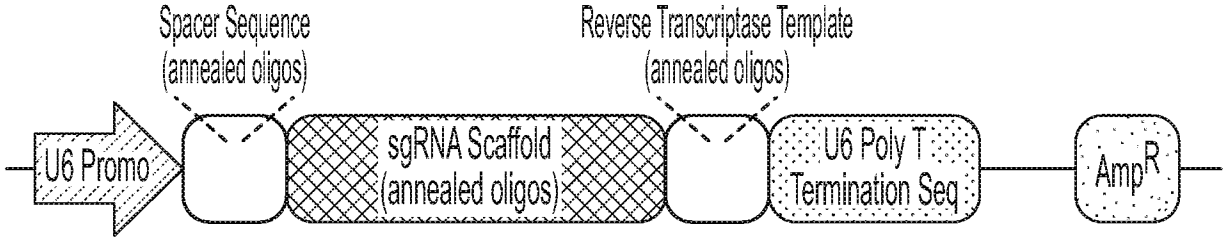


FIG. 54

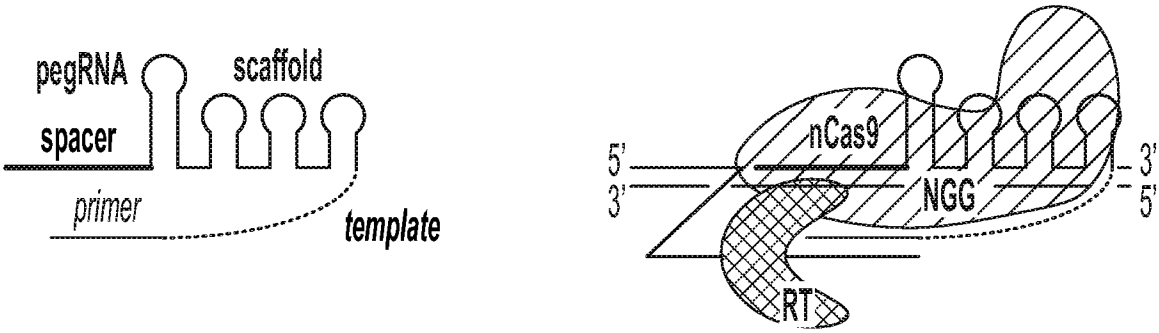


FIG. 55A

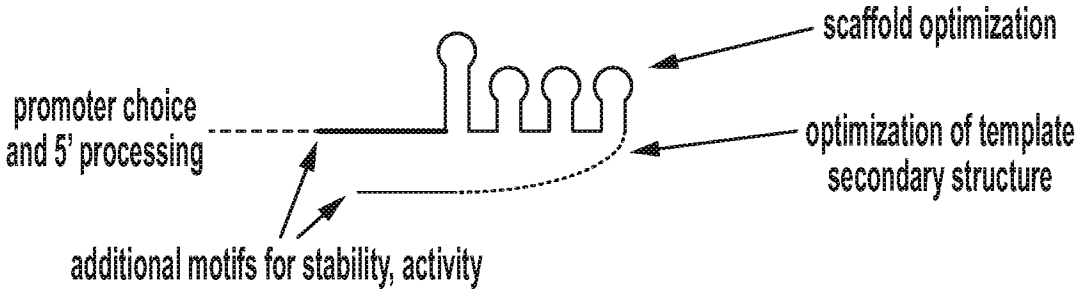


FIG. 55B

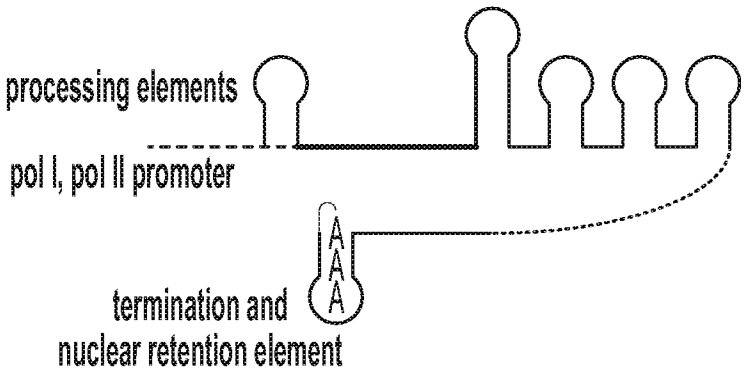


FIG. 55C

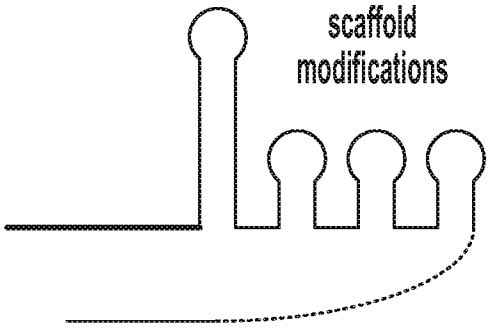


FIG. 55D

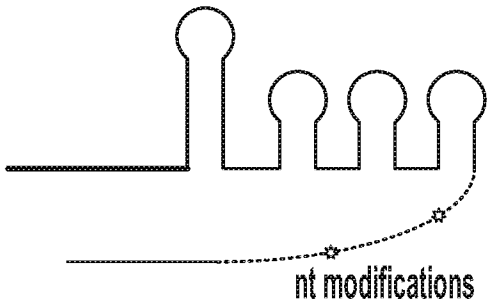


FIG. 55E

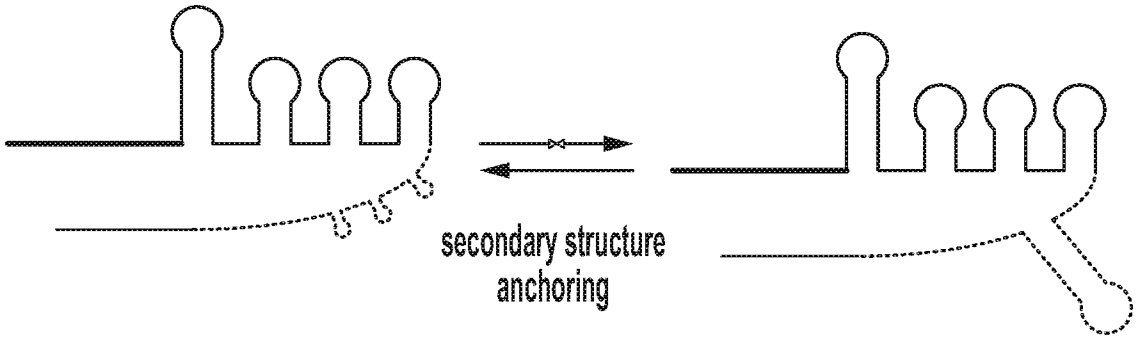


FIG. 55F

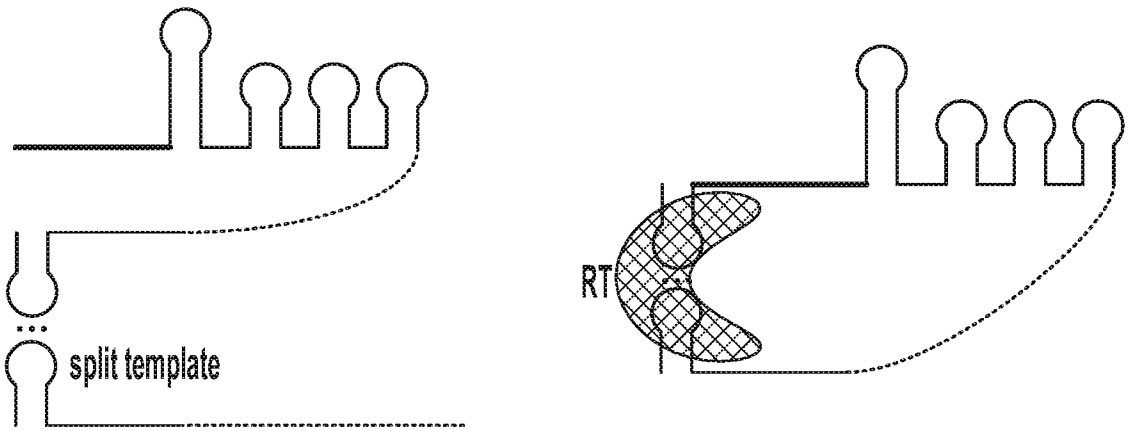


FIG. 55G

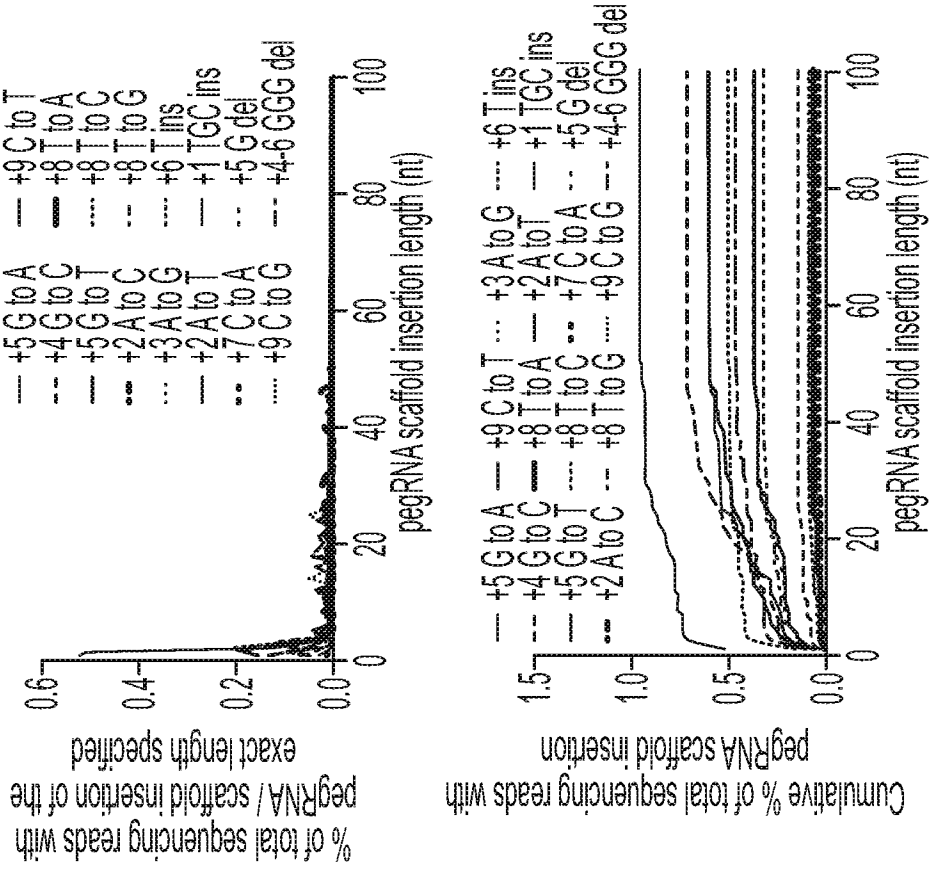
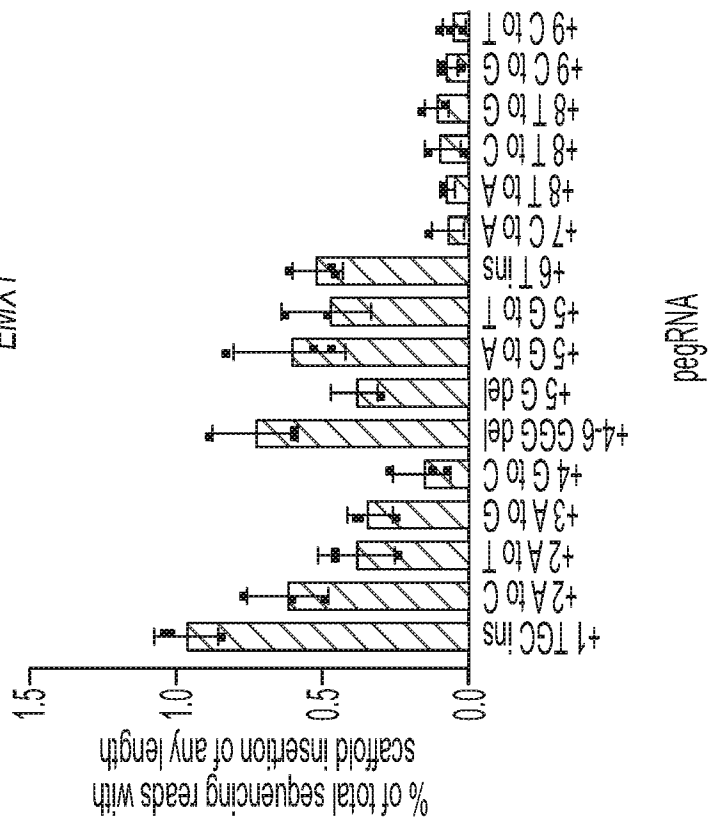

% of total sequencing reads with pegRNA / scaffold insertion of the exact length specified
pegRNA scaffold insertion length (nt)
— +5 G to A
-- +4 G to C
— +5 G to T
▪▪ +2 A to C
... +3 A to G
— +2 A to T
▪ +7 C to A
.... +9 C to G
— +9 C to T
-- +8 T to A
... +8 T to C
-- +8 T to G
... +6 T ins
— +1 TGC ins
- - +5 G del
-- +4-6 GGG del
Cumulative % of total sequencing reads with pegRNA scaffold insertion
pegRNA scaffold insertion length (nt)
— +5 G to A
-- +4 G to C
— +5 G to T
▪▪ +2 A to C
— +9 C to T
-- +8 T to A
--- +8 T to C
-- +8 T to G
... +3 A to G
— +2 A to T
▪ +7 C to A
.... +9 C to G
... +6 T ins
— +1 TGC ins
- - +5 G del
-- +4-6 GGG del

FIG. 56A


EMX1
% of total sequencing reads with scaffold insertion of any length
pegRNA
+1 TGC ins
+2 A to C
+2 A to T
+3 A to G
+4 G to C
+4-6 GGG del
+5 G del
+5 G to A
+5 G to T
+6 T ins
+7 C to A
+8 T to A
+8 T to C
+8 T to G
+9 C to G
+9 C to T

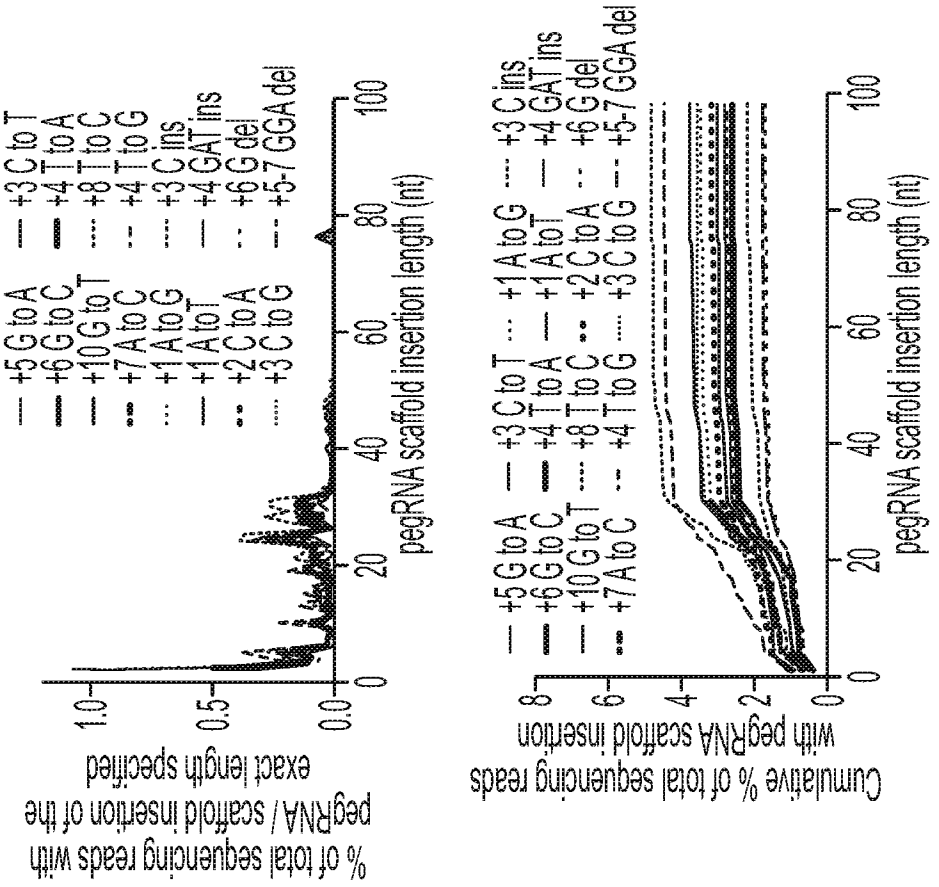
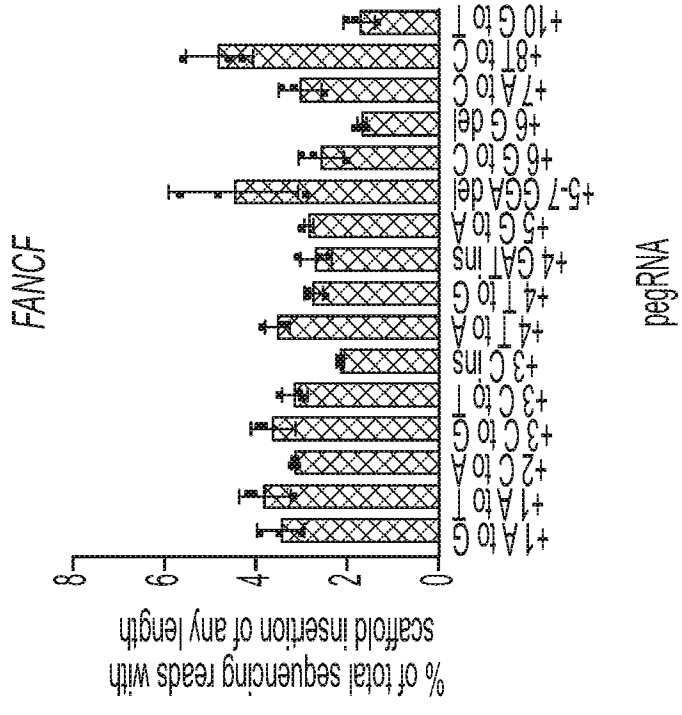

% of total sequencing reads with pegRNA / scaffold insertion of the exact length specified
pegRNA scaffold insertion length (nt)
— +5 G to A
— +6 G to C
— +10 G to T
— +7 A to C
— +1 A to G
— +1 A to T
— +2 C to A
— +3 C to G
— +3 C to T
— +4 T to A
— +8 T to C
— +4 T to G
— +3 C ins
— +4 GAT ins
— +6 G del
— +5-7 GGA del
Cumulative % of total sequencing reads with pegRNA scaffold insertion
pegRNA scaffold insertion length (nt)
— +5 G to A
— +6 G to C
— +10 G to T
— +7 A to C
— +3 C to T
— +4 T to A
— +8 T to C
— +4 T to G
— +1 A to G
— +1 A to T
— +2 C to A
— +3 C to G
— +3 C ins
— +4 GAT ins
— +6 G del
— +5-7 GGA del

FIG. 56B


% of total sequencing reads with scaffold insertion of any length
FANCF
pegRNA
+1A to G
+1A to T
+2C to A
+3C to G
+3C to T
+3C ins
+4T to A
+4T to G
+4 GAT ins
+5G to A
+5-7 GGA del
+6G to C
+6 G del
+7A to C
+8T to C
+10 G to T

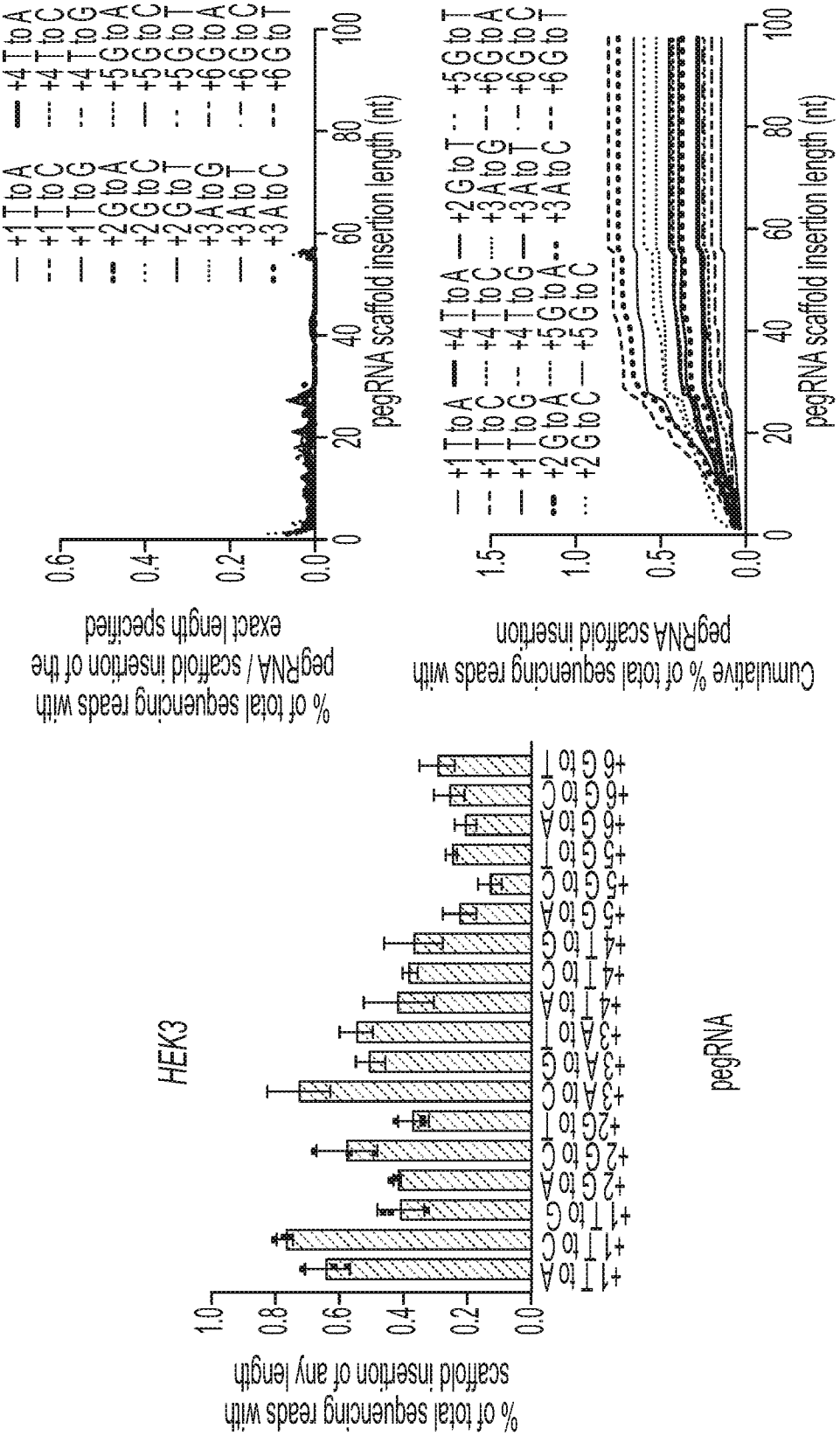

HEK3
% of total sequencing reads with scaffold insertion of any length
pegRNA
+1T to A
+1T to C
+1T to G
+2G to A
+2G to C
+2G to T
+3A to G
+3A to T
+3A to C
+4T to A
+4T to C
+4T to G
+5G to A
+5G to C
+5G to T
+6G to A
+6G to C
+6G to T
% of total sequencing reads with pegRNA / scaffold insertion of the exact length specified
pegRNA scaffold insertion length (nt)
Cumulative % of total sequencing reads with pegRNA scaffold insertion
pegRNA scaffold insertion length (nt)

FIG. 56C

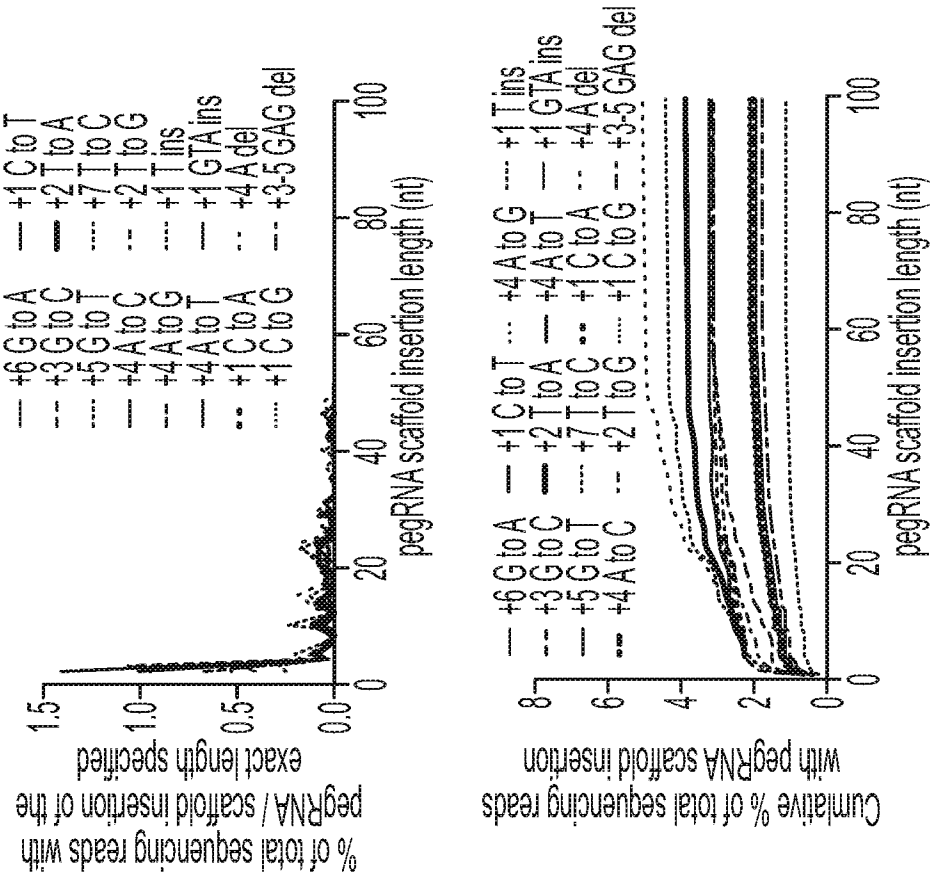
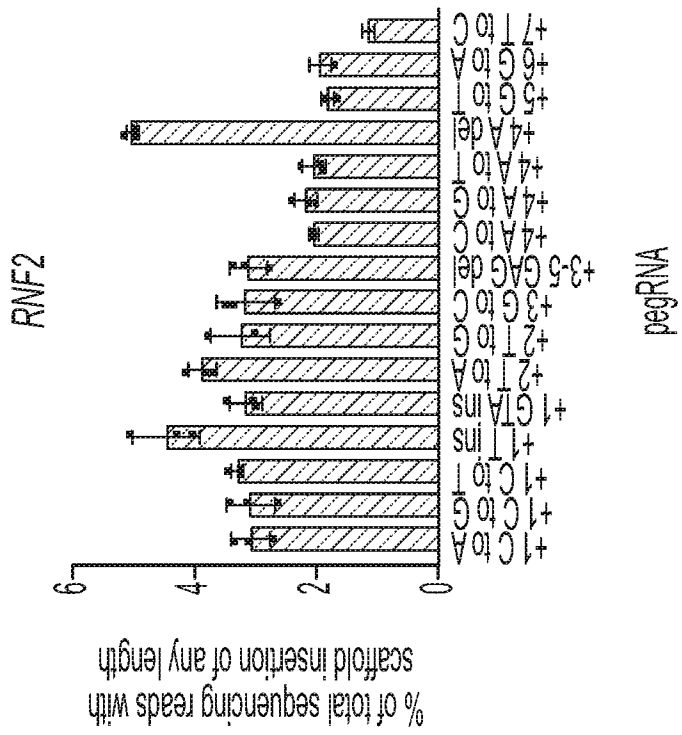

% of total sequencing reads with pegRNA / scaffold insertion of the exact length specified
pegRNA scaffold insertion length (nt)
— +6 G to A
-- +3 G to C
.... +5 G to T
— +4 A to C
-- +4 A to G
— +4 A to T
-•- +1 C to A
.... +1 C to G
— +1 C to T
-- +2 T to A
.... +7 T to C
-- +2 T to G
.... +1 T ins
— +1 GTA ins
-•- +4 A del
-•• +3-5 GAG del
Cumlative % of total sequencing reads with pegRNA scaffold insertion
pegRNA scaffold insertion length (nt)
— +6 G to A
-- +3 G to C
— +5 G to T
-•- +4 A to C
-•• +4 A to G
— +4 A to T
.... +1 C to A
-- +1 C to G
— +1 C to T
-- +2 T to A
— +7 T to C
.... +2 T to G
— +1 T ins
-- +1 GTA ins
-•- +4 A del
-•• +3-5 GAG del

FIG. 56D


% of total sequencing reads with scaffold insertion of any length
RNF2
pegRNA
+1 C to A
+1 C to G
+1 C to T
+1 T ins
+1 GTA ins
+2 T to A
+2 T to G
+3 G to C
+3-5 GAG del
+4 A to C
+4 A to G
+4 A to T
+4 A del
+5 G to T
+6 G to A
+7 T to C

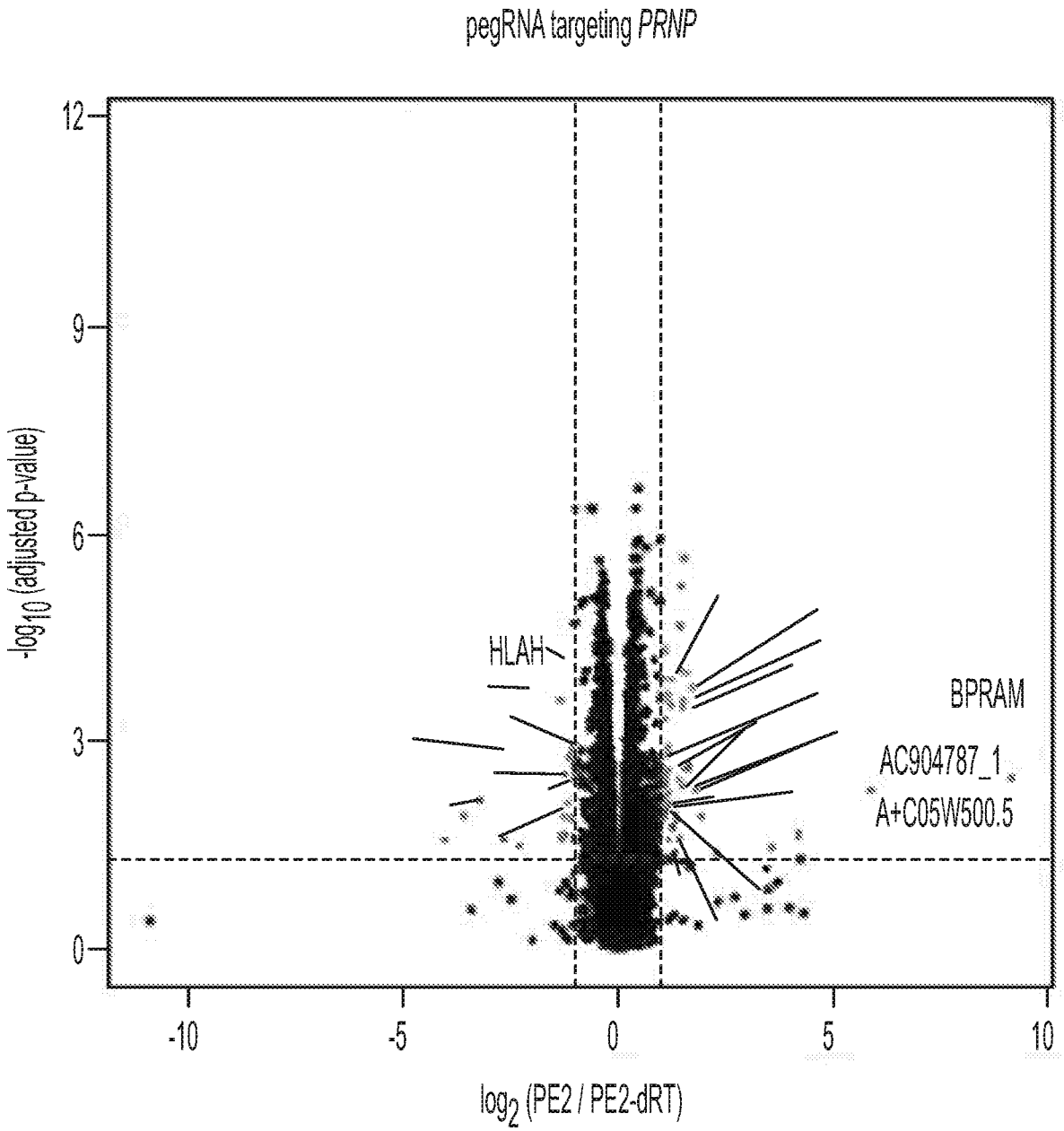

pegRNA targeting *PRNP*
12
9
6
3
0
-log10 (adjusted p-value)
HLAH
BPRAM
AC904787_1
A+C05W500.5
-10
-5
0
5
10
log2 (PE2 / PE2-dRT)

FIG. 57A

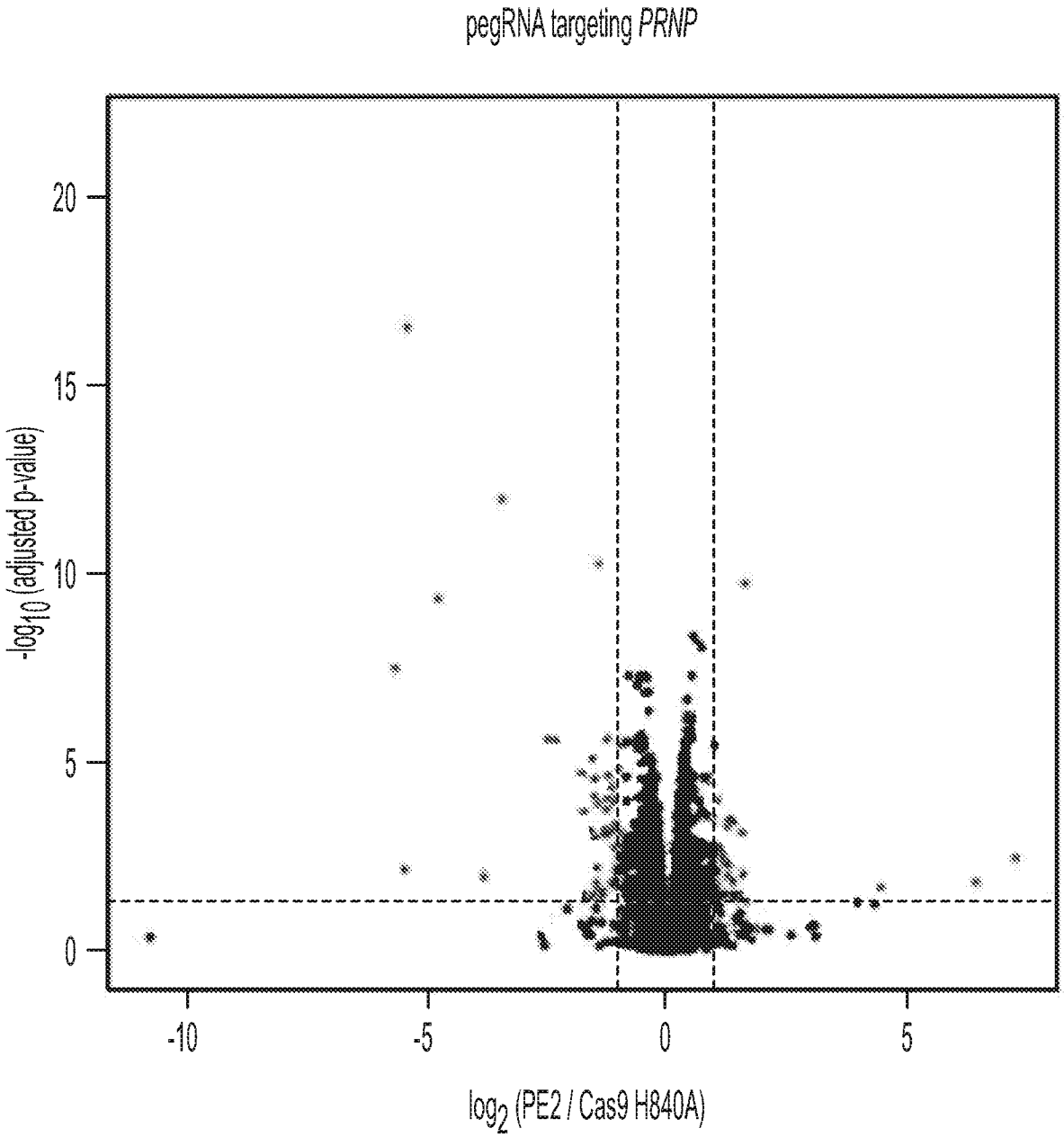

pegRNA targeting PRNP
20
15
10
5
0
-log10 (adjusted p-value)
-10
-5
0
5
log2 (PE2 / Cas9 H840A)

FIG. 57B

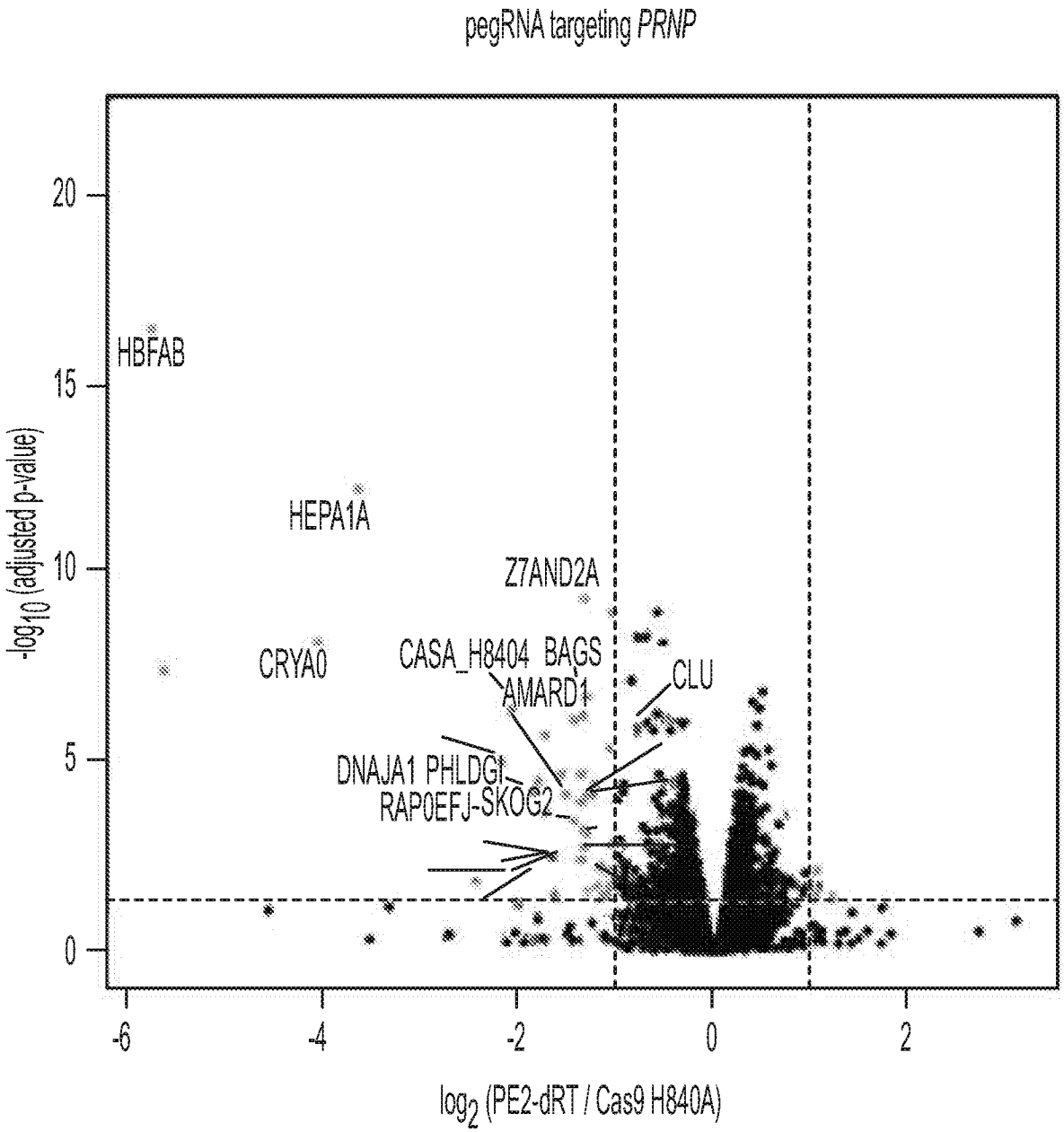

pegRNA targeting *PRNP*
HBFAB
HEPA1A
Z7AND2A
CRYAB
CASA_H8404
BAGS
AMARD1
CLU
DNAJA1
PHLDGI
RAP0EFJ-SKOG2
20
15
10
5
0
-6
-4
-2
0
2
$-\log_{10}$ (adjusted p-value)
$\log_2$ (PE2-dRT / Cas9 H840A)

FIG. 57C

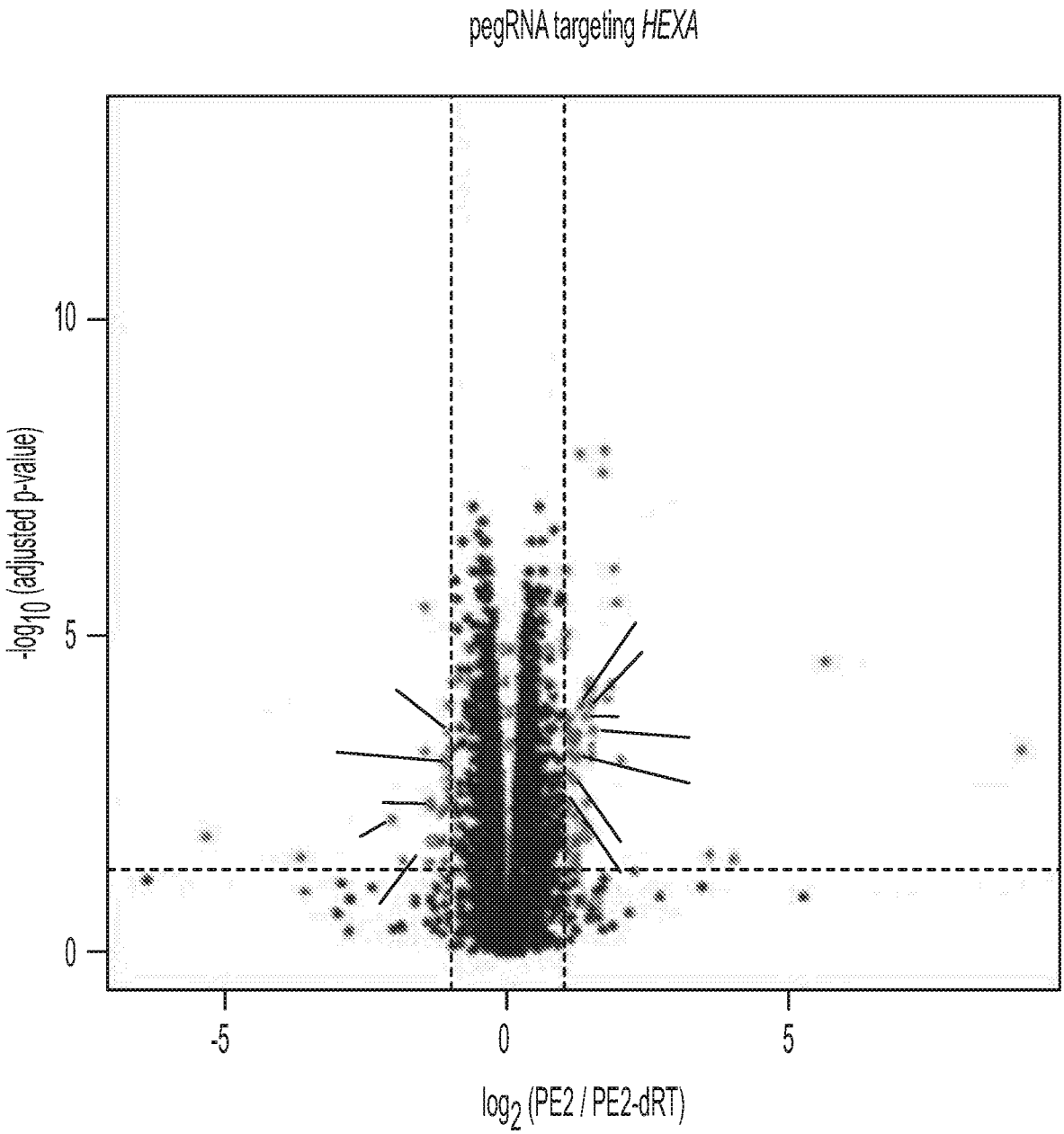

pegRNA targeting *HEXA*
10
5
0
-log10 (adjusted p-value)
-5
0
5
log2 (PE2 / PE2-dRT)

FIG. 57D

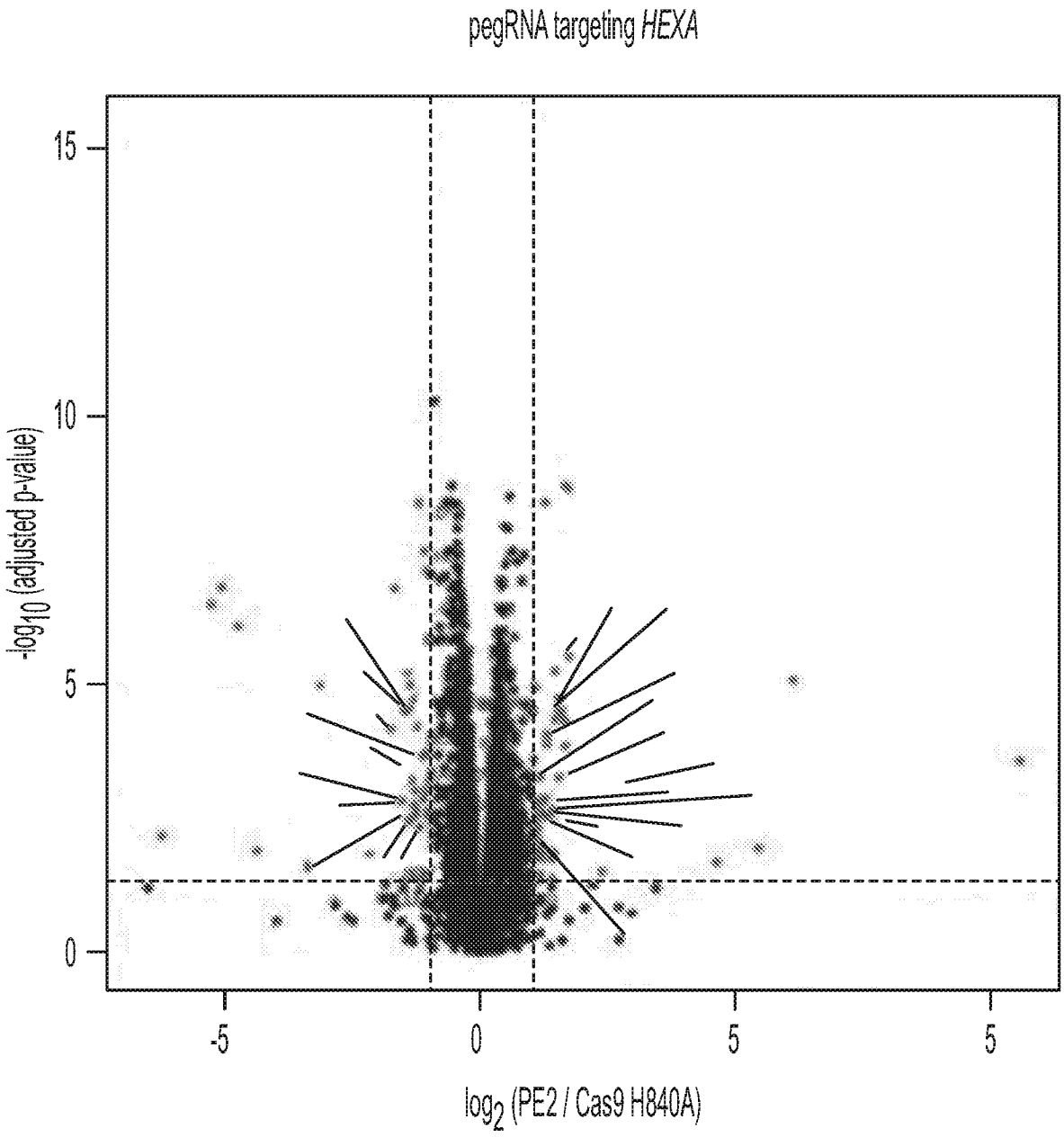

pegRNA targeting *HEXA*
15
10
5
0
-log10 (adjusted p-value)
-5
0
5
5
log2 (PE2 / Cas9 H840A)

FIG. 57E

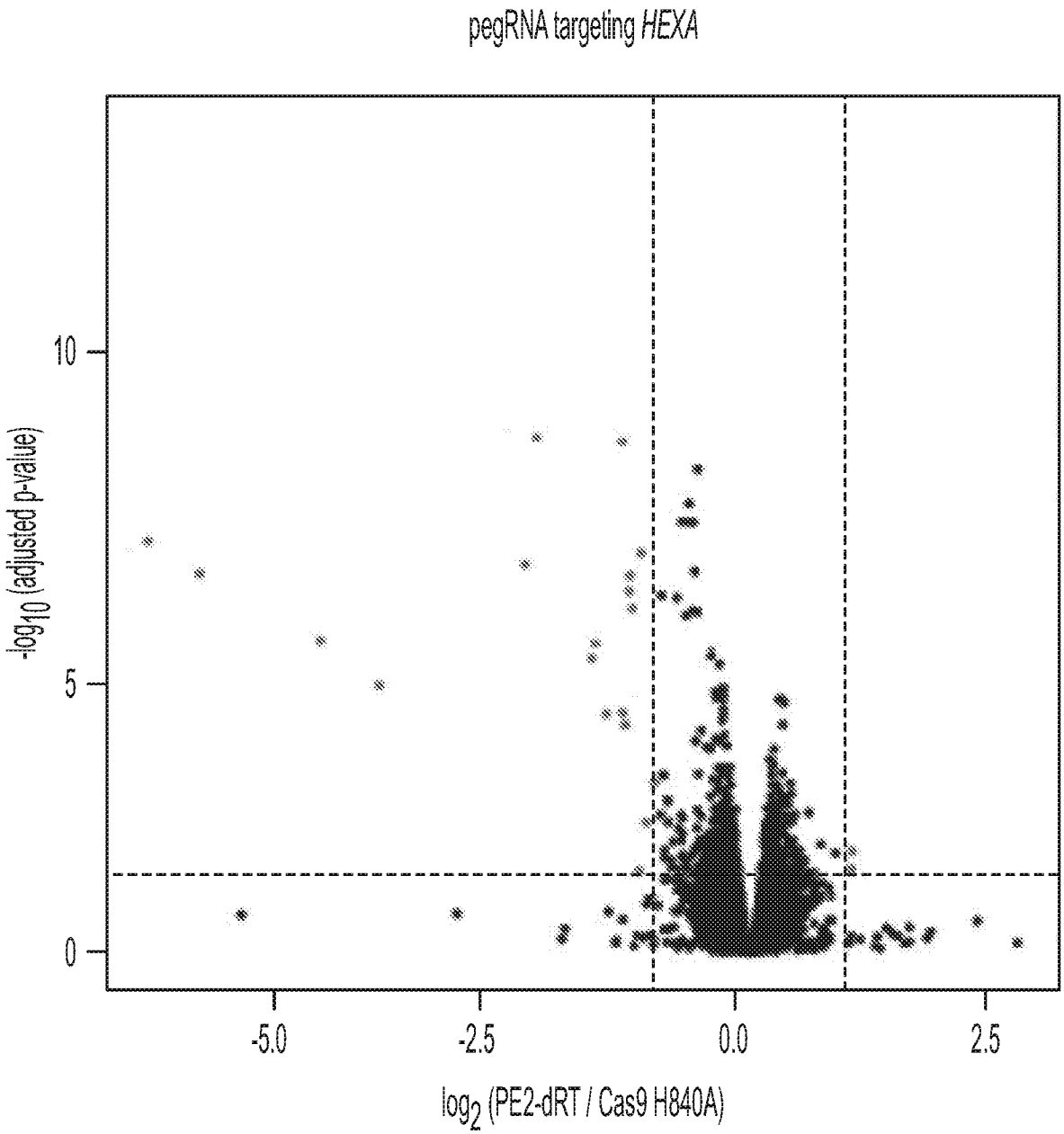

pegRNA targeting *HEXA*
10
5
0
$-\log_{10}$ (adjusted p-value)
-5.0
-2.5
0.0
2.5
$\log_2$ (PE2-dRT / Cas9 H840A)

FIG. 57F

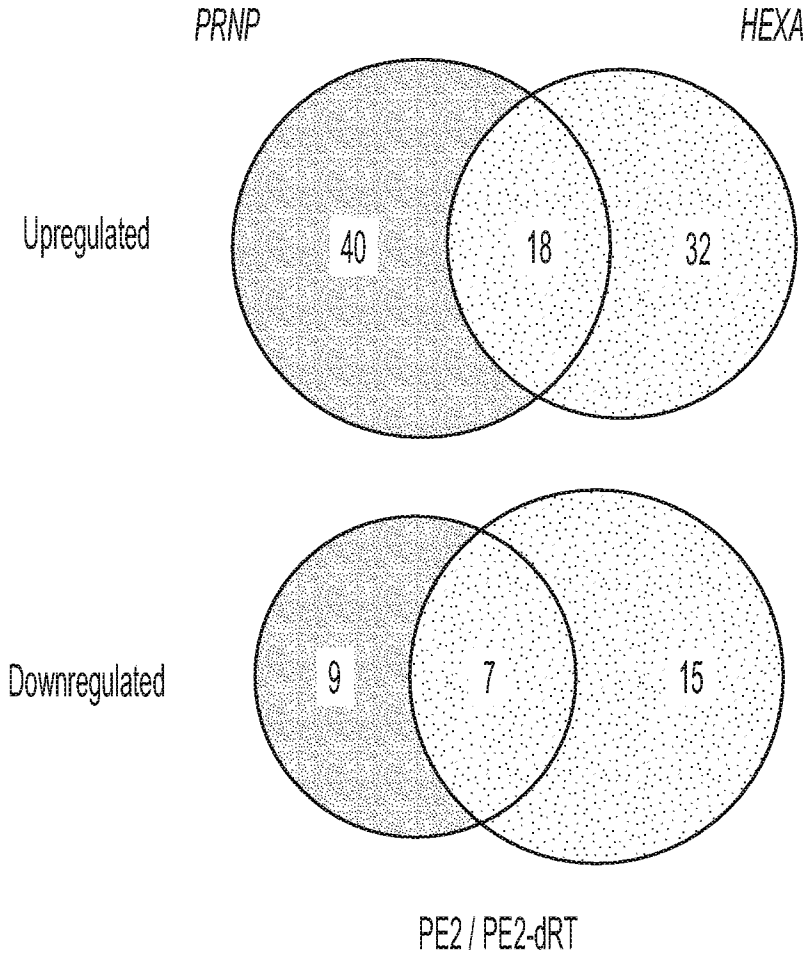

PRNP
HEXA
Upregulated
40
18
32
Downregulated
9
7
15
PE2 / PE2-dRT

FIG. 57G

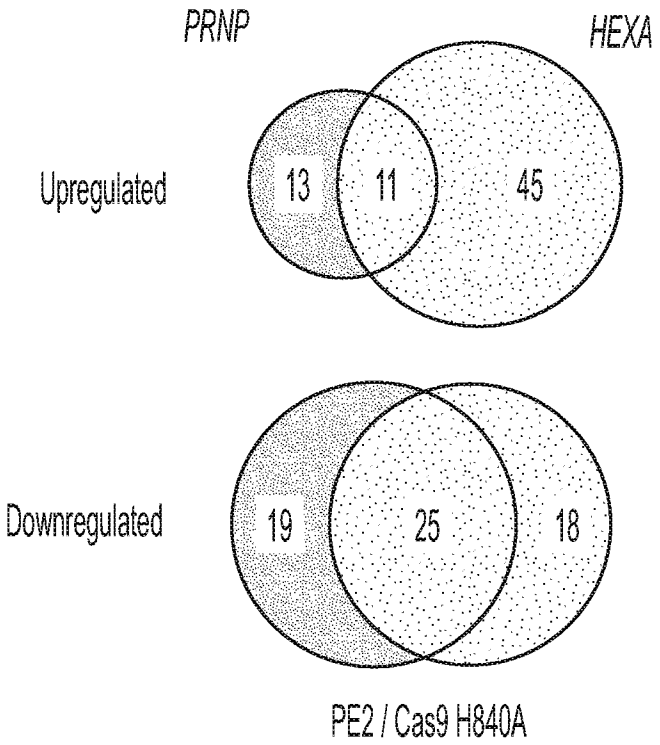


FIG. 57H

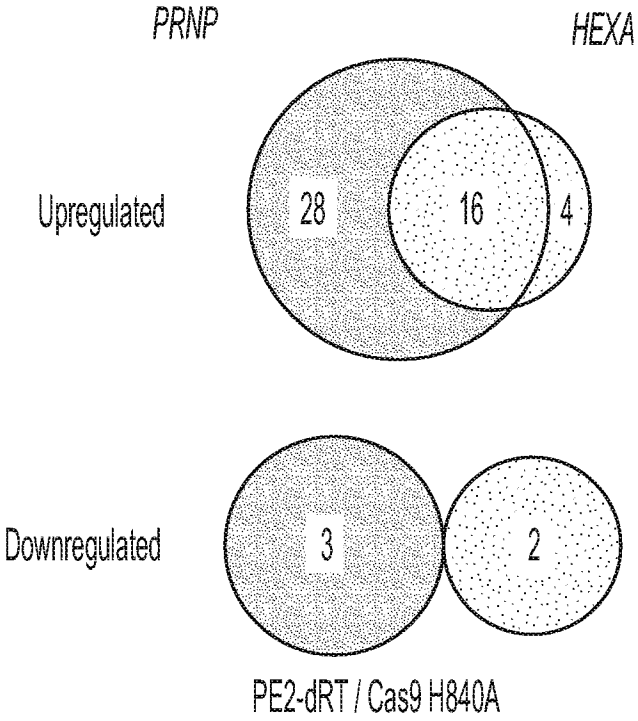


FIG. 57I

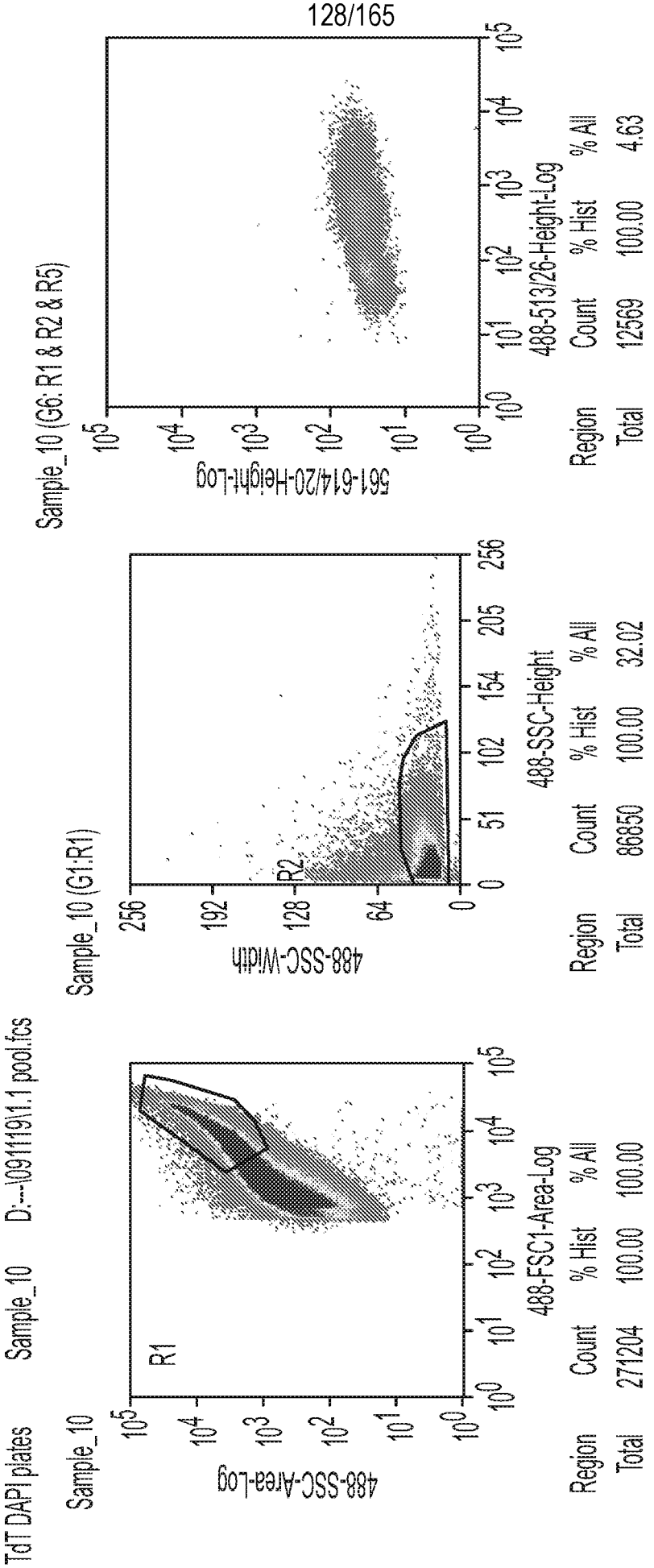
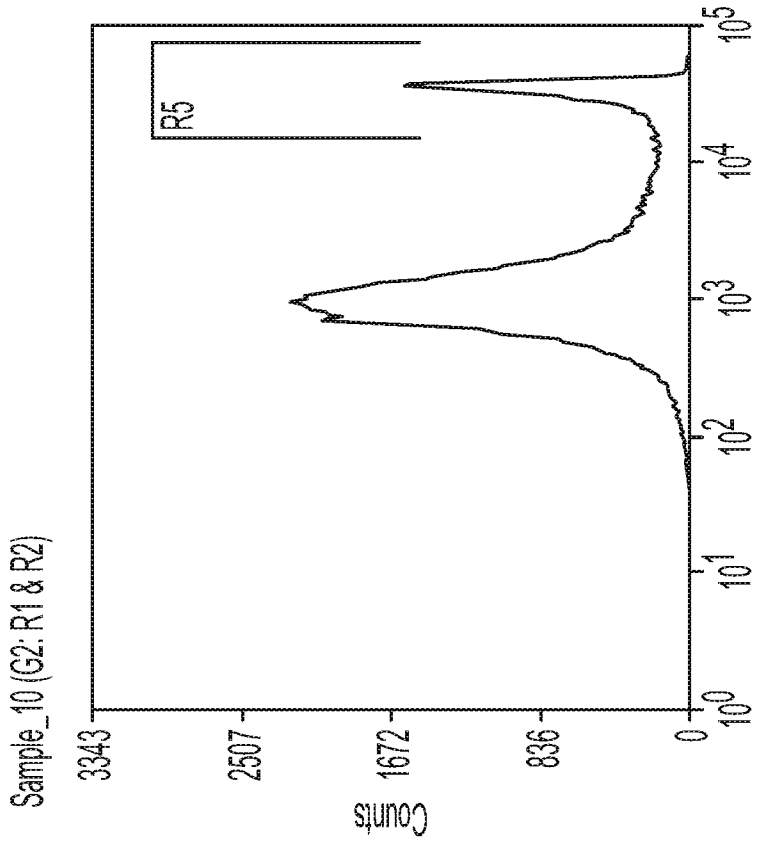
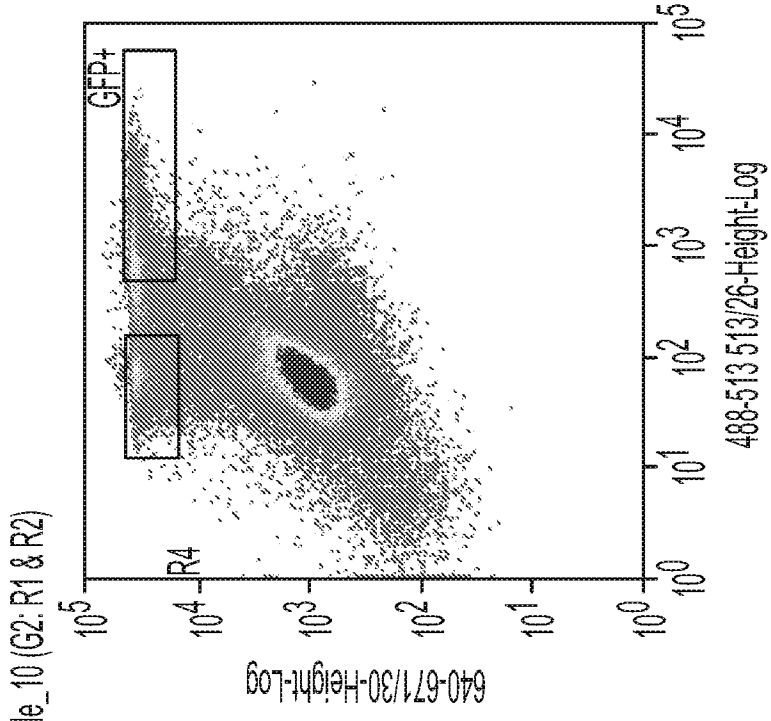

TdT DAPI plates
Sample_10
D:\...\091119\1.1 pool.fcs
Sample_10
488-SSC-Area-Log
R1
488-FSC1-Area-Log
Region Count % Hist % All
Total 271204 100.00 100.00
R1 86850 32.02 32.02
Sample_10 (G1:R1)
488-SSC-Width
R2
488-SSC-Height
Region Count % Hist % All
Total 86850 100.00 32.02
R2 81289 93.60 29.97
Sample_10 (G6: R1 & R2 & R5)
561-614/20-Height-Log
488-513/26-Height-Log
Region Count % Hist % All
Total 12569 100.00 4.63

FIG. 58



Region	Count	% Hist	Mean	CV
Total	81289	100.00	6521.68	179.64
R5	12569	15.46	32516.62	24.27



Region	Count	% Hist	% All	Bounds	Mode Count
Total	81289	100.00	29.97	(1.00,1000000.04) (100000.04,1...	128
GFP +	4418	5.43	1.63	(485.60, 48559.55) (58170.94,1...	40
R4	4709	5.79	1.74	(11.98,46415.91) (150.13,1501...	34

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FIG. 58
CONTINUED

Cloning overview

1. **Digest pU6-pegRNA-GG-Vector plasmid (component 1)** with *B*sal and isolate the plasmid fragment (~2.2kb) containing the origin of replication, U6 promoter, U6 poly-T termination sequence, and Amp^R gene
2. **Order oligonucleotides for:**
   a. The desired spacer (target) sequence flanked by indicated overhangs (**component 2**)
      i. Use the desired target's 5'-3' sequence for the top strand oligonucleotide (including the 5' CACC and 3' GTTTT overhangs) and use the reverse complement of the target sequence for the bottom strand oligonucleotide (including the 5' CTCTAAAAC overhang). Spacer sequences must begin with a G nucleotide for efficient transcription initiation.
   b. The desired pegRNA 3' extension template flanked by the indicated overhangs (**component 3**)
      i. Use the RNA sense sequence as the top strand oligonucleotide (featuring the 5' GTGC overhang) and use the reverse complement of this sequence for the bottom strand oligonucleotide (featuring the 5' AAAA overhang).
   c. SpCas9 sgRNA scaffold sequence featuring compatible golden gate overhangs (**component 4**)
      i. These oligonucleotides are not the complete scaffold sequence, as overhangs from the remaining components contribute several missing nucleotides
      ii. Note: these oligonucleotides must be 5' phosphorylated. Oligonucleotides can be 5' phosphorylated by the manufacturer or 5' phosphorylated enzymatically using T4 PNK (see protocol below)
3. **Anneal top and bottom oligonucleotides** for components 2, 3, and 4 in separate annealing reactions according to the protocol below. If the SpCas9 sgRNA scaffold sequence (component 4) was not phosphorylated, phosphorylate with T4 PNK.
4. **Golden Gate assembly of isolated 2.2-kb fragment from component 1 with components 2, 3, and 4**
5. **Transform the ligation product into *E. coli*.** The antibiotic resistance conferred by component 1 from the pU6- pegRNA-GG-vector plasmid is ampicillin and carbenicillin resistance.
6. **Isolate and sequence** plasmids from the resulting clonal transformants

FIG. 59A

pegRNA cloning protocol

Step 1: Digest pU6-pegRNA-GG-Vector plasmid (component 1)

Combine the following in a PCR tube:

2000 ng pU6-pegRNA-GG-Vector (component 1)	X µL
Bsa1-HFv2 (NEB)	1.0 µL
10x Cutsmart Buffer	3.0 µL
H_2O	to 30.0 µL
Total reaction volume	30.0 µL

Incubate at 37 °C for 4-16 hours
Isolate ~2.2-kb fragment from cut plasmid.

FIG. 59B

Steps 2 and 3: Order and anneal oligonucleotide parts (components 2, 3, and 4)

Materials

Annealing buffer: H_2O supplemented with 10 mM Tris-Cl pH 8.5 and 50 mM NaCl
Complementary oligonucleotide pairs

Protocol

Combine the following in a PCR tube:

Top oligonucleotide, 100 µM	1.0 µL
Bottom oligonucleotide, 100 µM	1.0 µL
Annealing buffer (components 2, 3, and 4)	23.0 µL
Total reaction volume	25.0 µL

In thermocycler, heat at 95 °C for 3 minutes, then cool gradually (0.1 °C/s) to 22 °C

Dilute annealed oligonucleotides 1:4 by adding 75 µL H_2O. The final concentration of each oligonucleotide will be 1 µM after this dilution. Do not dilute the sgRNA scaffold (component 4) if phosphorylating by PNK in step 2.5.

FIG. 59C

Step 2.b.ii.: sgRNA scaffold phosphorylation (unnecessary if oligonucleotides were purchased phosphorylated)

Protocol

Combine the following in a PCR tube:

4 µM oligonucleotide duplex from step 1	6.25 µL
10x T4 DNA ligase buffer (NEB)	2.50 µL
T4 PNK (NEB)	0.50 µL
H_2O	15.75 µL
Total reaction volume	20.0 µL

In thermocycler, incubate at 37 °C for 60 minutes
Following this phosphorylation, annealed scaffold oligonucleotides are now at a concentration of 1 µM.
Proceed to step 3.

FIG. 59D

Step 4: peg RNA assembly

pegRNA, Golden Gate assembly reaction

Digested pU6-pegRNA-GG plasmid-vector - Pre-cut, isolated 2.2-kb fragment	1.00 µL @ 30 ng/µL
Annealed protospacer oligonucleotides (component 2)	1.00 µL @ 1 µM
Annealed pegRNA 3'-extension oligonucleotides (component 4)	1.00 µL @ 1 µM
sgRNA scaffold annealed oligonucleotides (component 3) - Oligonucleotides *must be phosphorylated*	1.00 µL @ 1 µM
*Bsa*I-HFv2 (NEB)	0.25 µL
T4 DNA ligase (NEB)	0.50 µL
10x T4 DNA ligase buffer (NEB)	1.00 µL
H_2O	4.25 µL
Total reaction volume:	10.0 µL

Incubate at room temperature for 10 min
Following incubation, perform the following program in a thermocycler:
5 min at 37 °C then 5 min at 80 °C then hold at 12 °C

FIG. 59E

Steps 5 and 6: Transformation of assembled plasmids

Transform 1 µL of the 10 µL assembly reaction into 10 µL of competent cells. The desired transformants will be resistant to ampicillin and carbenicillin.

The following diagram summarizes the pegRNA cloning protocol.

FIG. 59F

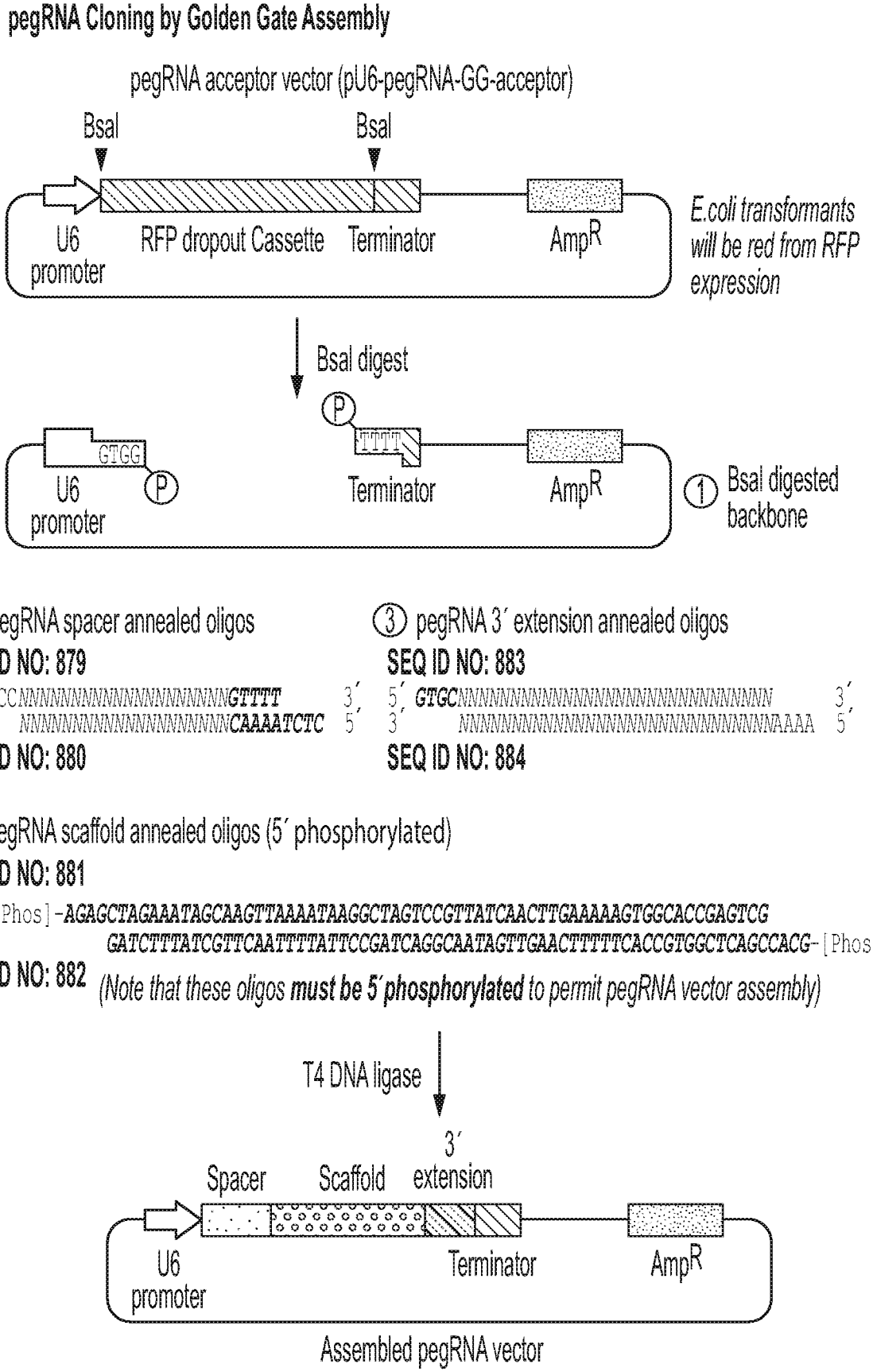

pegRNA Cloning by Golden Gate Assembly
pegRNA acceptor vector (pU6-pegRNA-GG-acceptor)
BsaI
BsaI
U6 promoter
RFP dropout Cassette
Terminator
AmpR
E.coli transformants will be red from RFP expression
BsaI digest
GTGG
P
U6 promoter
P
TTTT
Terminator
AmpR
1 BsaI digested backbone
2 pegRNA spacer annealed oligos
SEQ ID NO: 879
5′ CACCNNNNNNNNNNNNNNNNNNGTTTT 3′
3′ NNNNNNNNNNNNNNNNNNCAAAATCTC 5′
SEQ ID NO: 880
3 pegRNA 3′ extension annealed oligos
SEQ ID NO: 883
5′ GTGCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN 3′
3′ NNNNNNNNNNNNNNNNNNNNNNNNNNNNAAA 5′
SEQ ID NO: 884
4 pegRNA scaffold annealed oligos (5′ phosphorylated)
SEQ ID NO: 881
5′ [Phos]-AGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG 3′
3′ GATCTTTATCGTTCAATTTTATTCCGATCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCACG-[Phos] 5′
SEQ ID NO: 882
(Note that these oligos must be 5′ phosphorylated to permit pegRNA vector assembly)
T4 DNA ligase
3′ extension
Spacer
Scaffold
U6 promoter
Terminator
AmpR
Assembled pegRNA vector

FIG. 59G

```
## sgRNA scaffold sequence search ##

import pandas as pd
import Bio as bio
from Bio import SeqIO
import glob

#generates list of fastq files to analyze
sources = glob.glob('*.fastq')

#reads the fastq files into a dictionary with the file names as keys
fastqdict = {}
for i in range(len(sources)):
        temp = list(SeqIO.parse(source[i],"fastq"))
        fastqdict[sources[i]]= [str(temp[k].seq) for k in range(len(temp))]

#the referenced sequence to be searched for is entered into the following dictionary with
#an appropriate key
scaffdict =
{'HEK3':'CAGAGGACCGACTCGGTCCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACTCACGTGCTCAGTCTGGGCCGGTG', (SEQ ID NO: 885)
'EMX1':'ATCACGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TGCTATTTCTAGCTCTAAAACTTCTTCTTCTGCTCGGACTCGGTG', (SEQ ID NO: 886)
'FANCF':'TTTCCGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAAC
TTGCTATTTCTAGCTCTAAAACGGTGCTGCAGAAGGGATTCCGGTG', (SEQ ID NO: 887)
'RNF2':'TCGTTGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCAGGTAATGACTAAGATGACGGTG'} (SEQ ID NO: 888)

#matches and counts iterative slices of the reference string to the appropriate fastq files
#reference key must be contained in the name of the fastq file
#generated values represent cumulative counts for a minimum degree of sgRNA integration
#i.e. a given value x means x reads contain y or more bases of the scaffold
resultdict = dict.fromkeys(sources)
for key in fastqdict:
   for scaffold in scaffdict:
      if scaffold in str(key):
         resultlist = []
         for j in range(len(scaffdict[scaffold])):
```

FIG. 60A

```
                extent = scaffdict[scaffold][0:(j+1)]
                counter = 0
                for i in range(len(fastqdict[key])):
                    if extent in fastqdict[key][i]:
                        counter = counter + 1
                resultlist.append(counter)
            resultdict[key]=resultlist

#writes the results into a dataframe indexed from 1
resultdf = pd.DataFrame.from_dict(resultdict)
resultdf = resultdf.reindex(sorted(resultdf.columns), axis=1)
resultdf.index = range(1,len(resultdf)+1)

#converts the cumulative count values into specific counts
#i.e. a given value x means x reads contain exactly y bases of the scaffold
resultdf2=resultdf.copy()
for entry in resultdf:
    for i in range(1,len(resultdf[entry])+1):
        try:
            resultdf2[entry][i] = resultdf[entry][i]-resultdf[entry][i+1]
        except:
            resultdf2[entry][i] = resultdf[entry][i]

#converts the specific counts values into frequencies
resultdf3=resultdf2.copy()
for entry in resultdf3:
    resultdf3[entry]=resultdf2[entry].div(resultdf[entry][1])*100

#reads the results into excel files
resultdf.to_excel('cumulativecounts.xlsx')
resultdf2.to_excel('specificcounts.xlsx')
resultdf3.to_excel('specificfrequencies.xlsx')
```

FIG. 60B

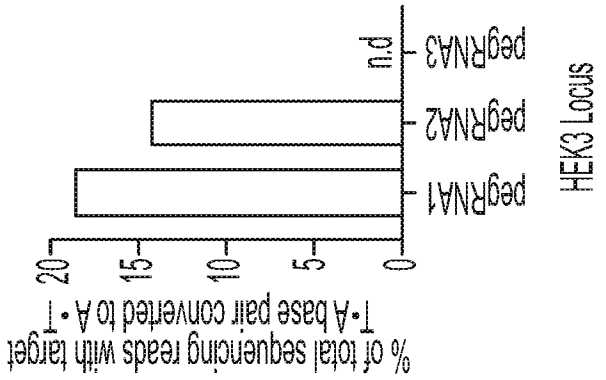


FIG. 62A

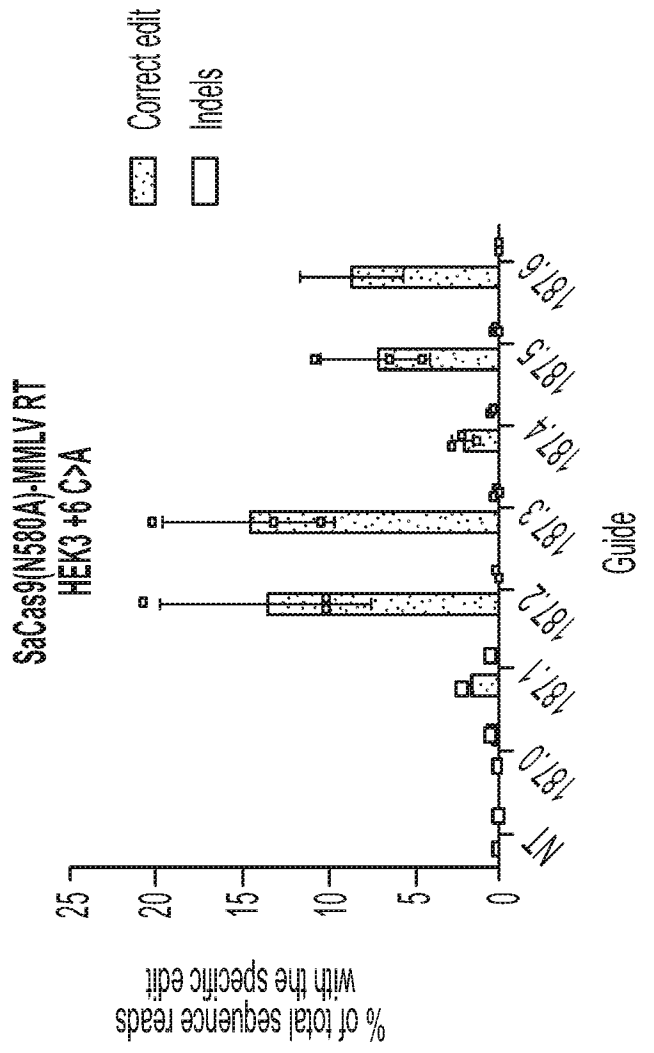


FIG. 61

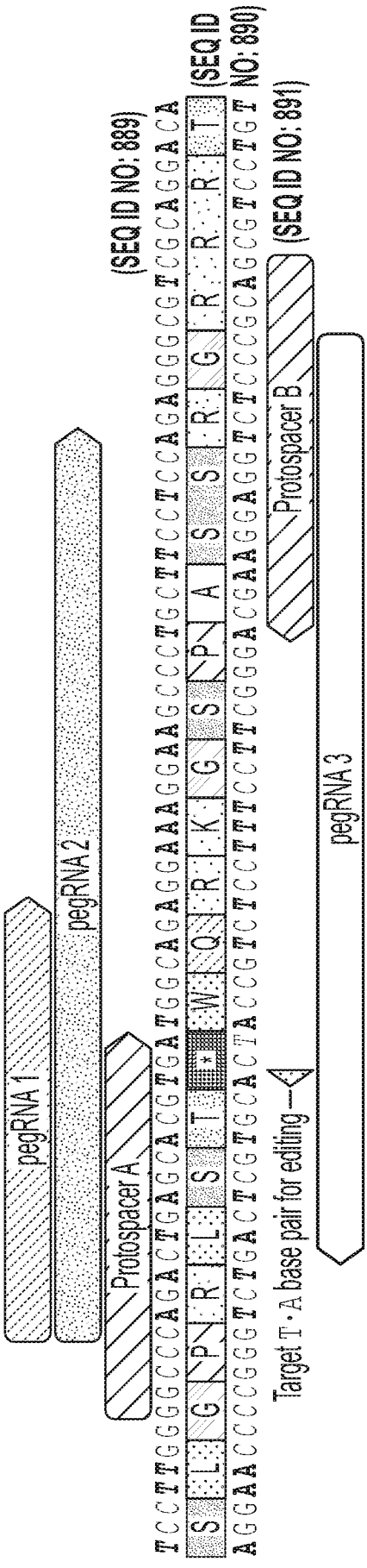

pegRNA 1
pegRNA 2
Protospacer A
Target T · A base pair for editing
pegRNA 3
Protospacer B
(SEQ ID NO: 889)
(SEQ ID NO: 890)
(SEQ ID NO: 891)

FIG. 62B

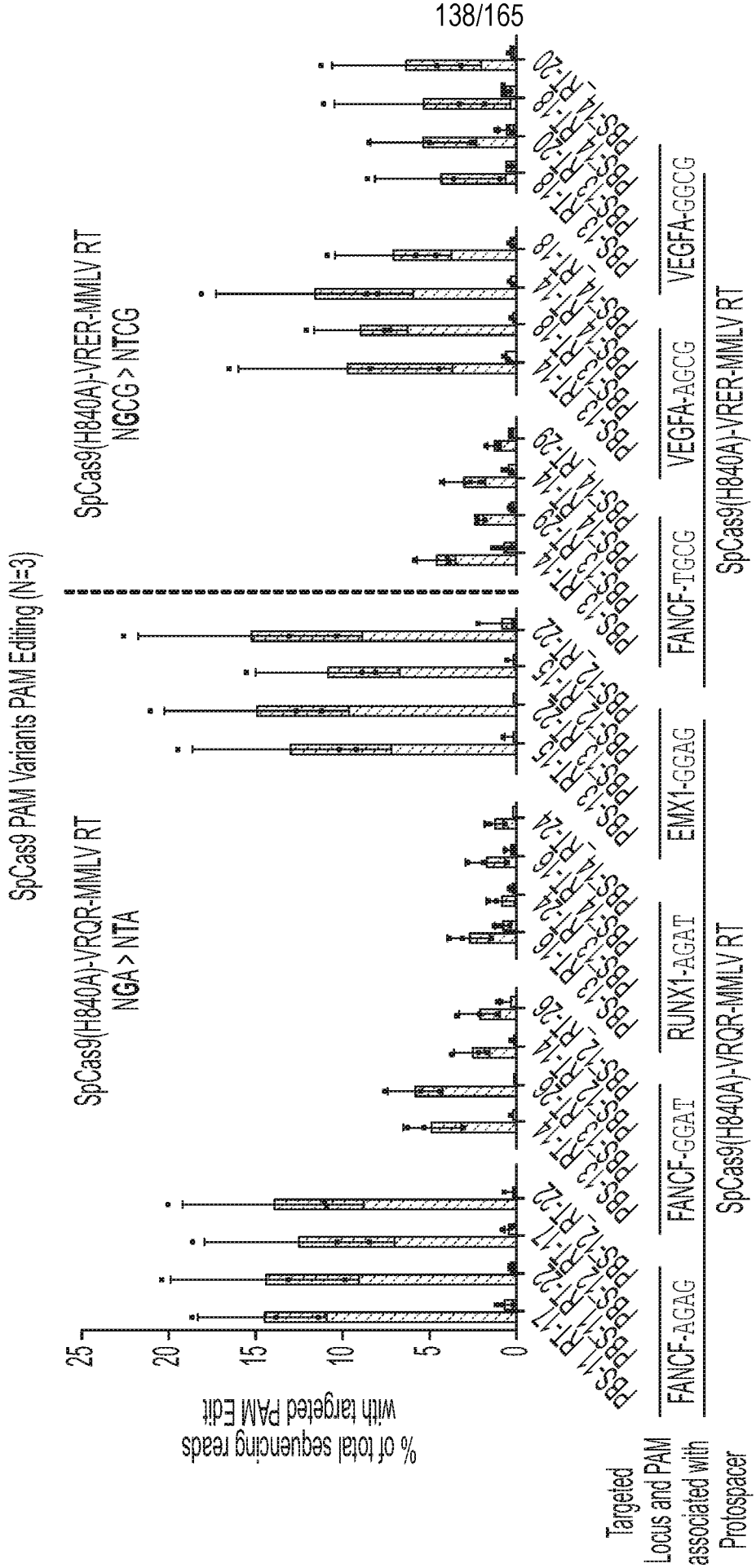

SpCas9 PAM Variants PAM Editing (N=3)
SpCas9(H840A)-VRQR-MMLV RT
NGA > NTA
SpCas9(H840A)-VRER-MMLV RT
NGCG > NTCG
% of total sequencing reads with targeted PAM Edit
25
20
15
10
5
0
PBS13_RT17
PBS13_RT20
PBS13_RT22
PBS13_RT26
PBS13_RT14
PBS13_RT16
PBS13_RT24
PBS13_RT15
PBS13_RT22
PBS13_RT29
PBS13_RT14
PBS13_RT18
PBS13_RT20
PBS13_RT18
PBS14_RT20
Targeted Locus and PAM associated with Protospacer
FANCF-AGAG
FANCF-GGAT
RUNX1-AGAT
EMX1-GGAG
FANCF-TGCG
VEGFA-AGCG
VEGFA-GGCG
SpCas9(H840A)-VRQR-MMLV RT
SpCas9(H840A)-VRER-MMLV RT
pegRNA Primer Binding Site (PBS) Length and RT Template (RT) Length and Prime Editing System Used
Percent G>T Editing From Total Reads
Indels from total reads

FIG. 63

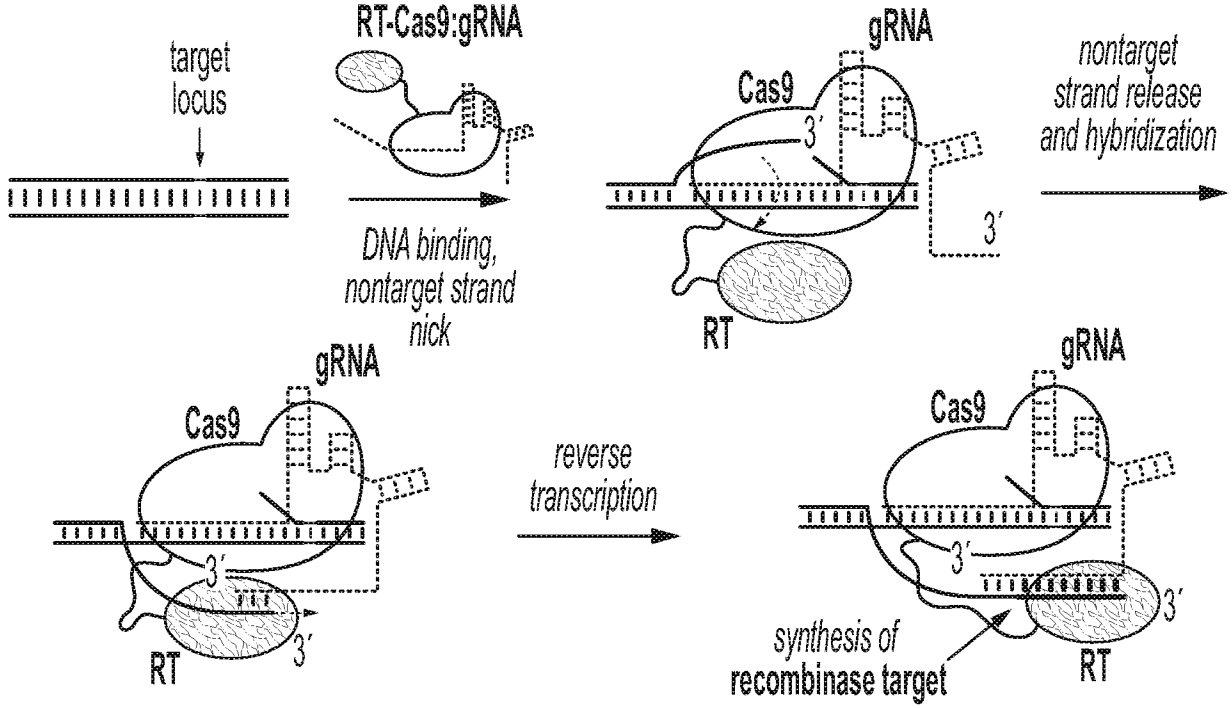


FIG. 64A

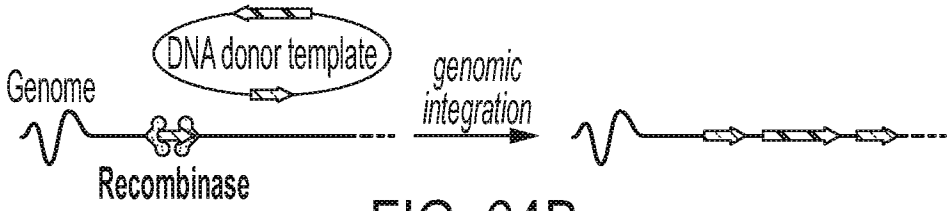


FIG. 64B

FIG. 64C

FIG. 64D

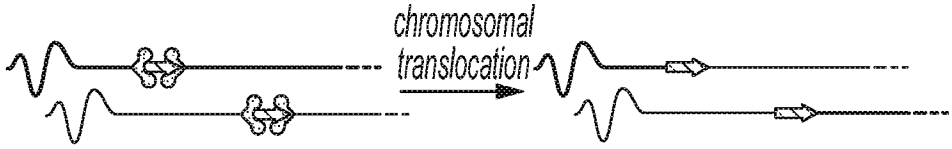


FIG. 64E

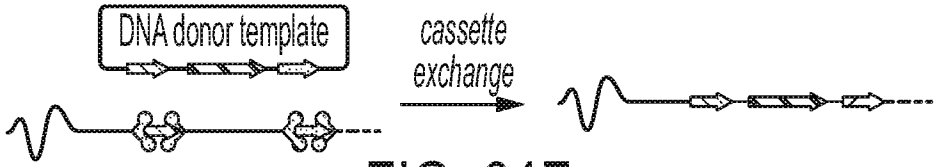


FIG. 64F

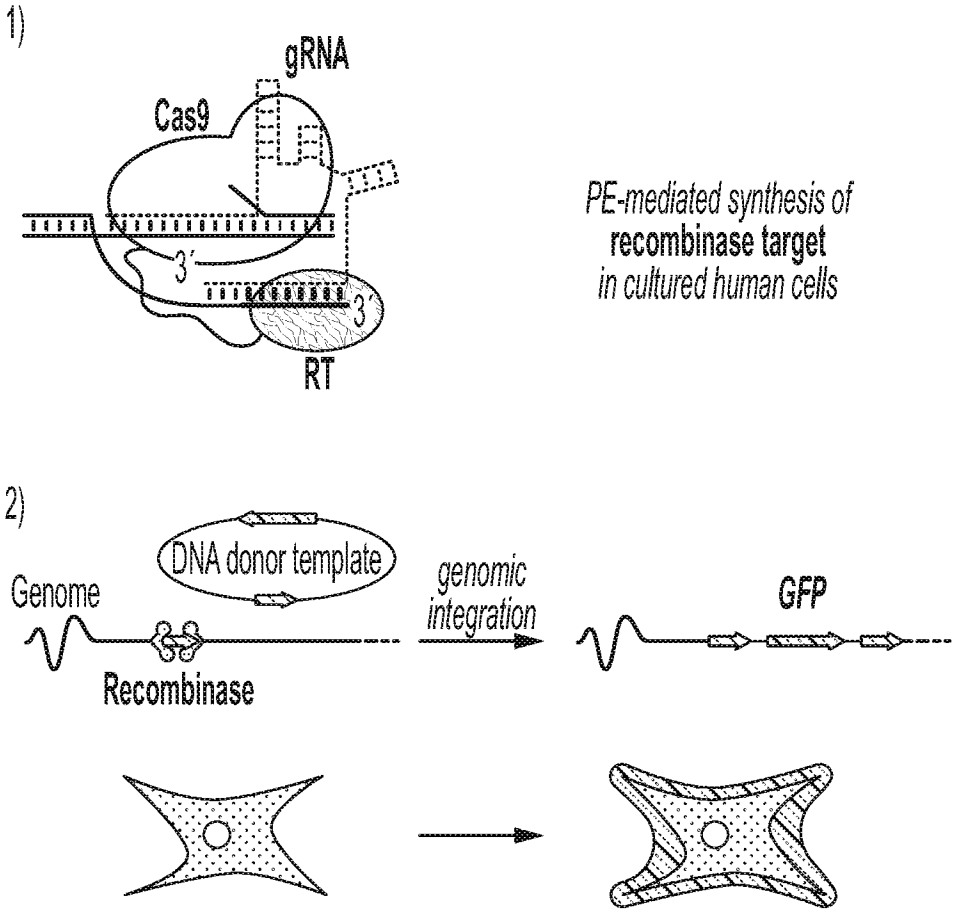

1)
gRNA
Cas9
3'
3'
RT
PE-mediated synthesis of
recombinase target
in cultured human cells
2)
DNA donor template
Genome
genomic
integration
GFP
Recombinase

FIG. 65

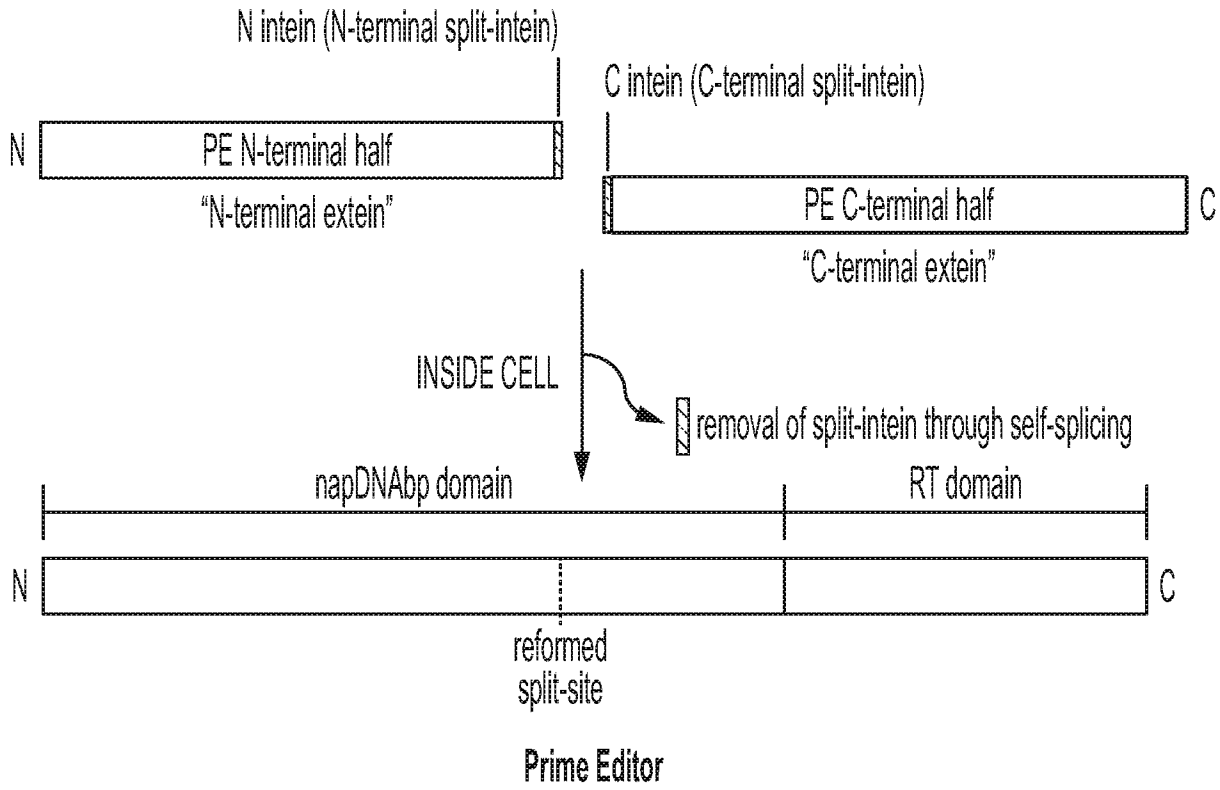

N intein (N-terminal split-intein)
C intein (C-terminal split-intein)
N
PE N-terminal half
"N-terminal extein"
PE C-terminal half
C
"C-terminal extein"
INSIDE CELL
removal of split-intein through self-splicing
napDNAbp domain
RT domain
N
C
reformed
split-site
Prime Editor

FIG. 66

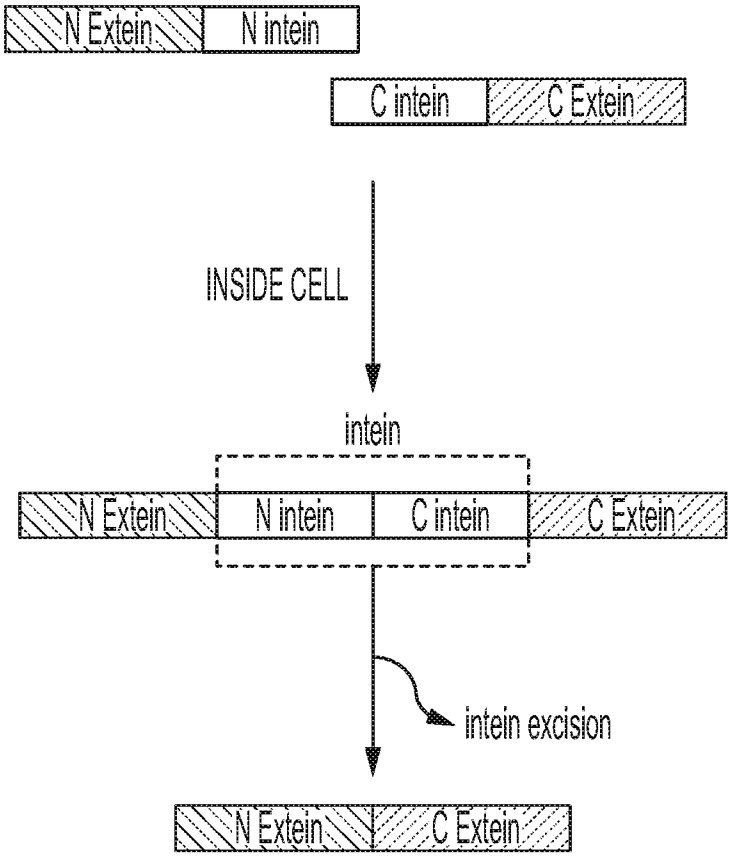

N Extein
N intein
C intein
C Extein
INSIDE CELL
intein
N Extein
N intein
C intein
C Extein
intein excision
N Extein
C Extein

FIG. 67A

intein excision mechanism

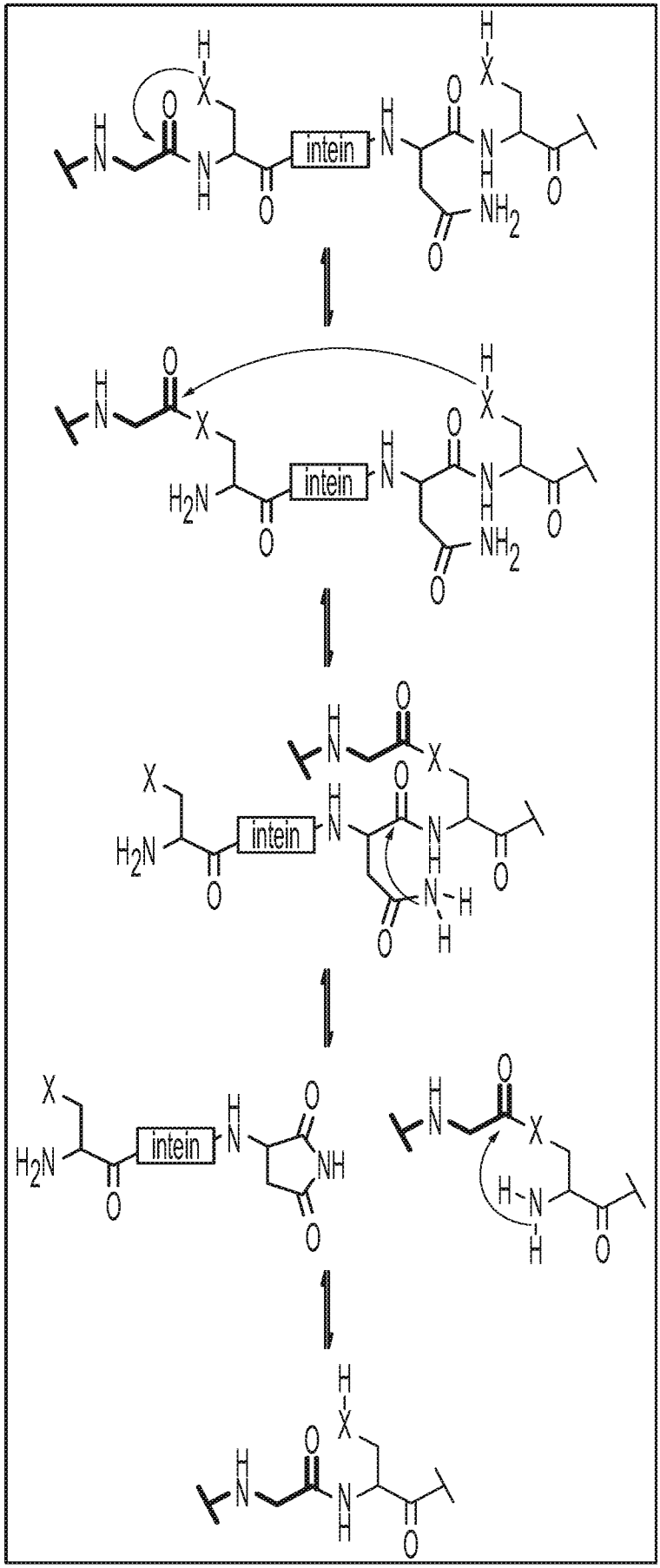


FIG. 67B

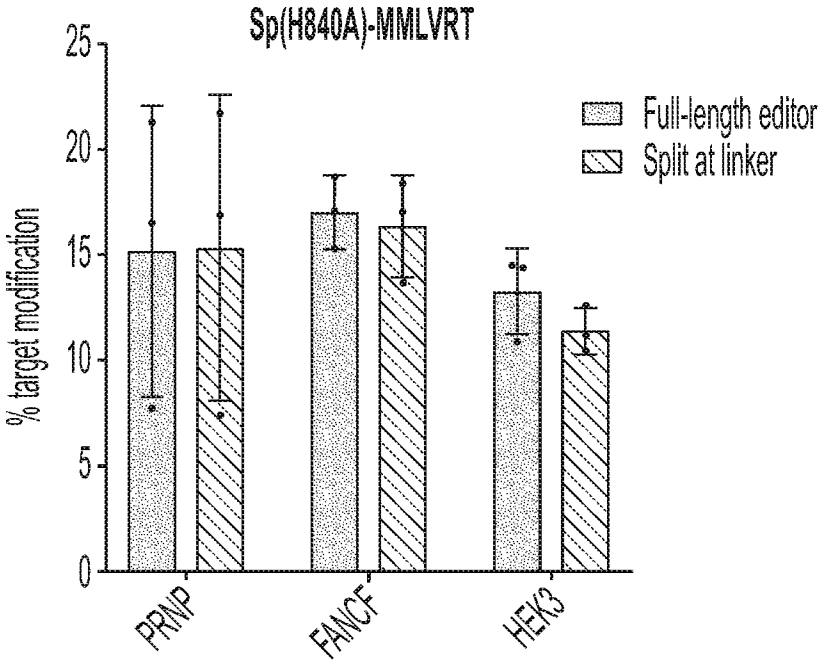


FIG. 68A

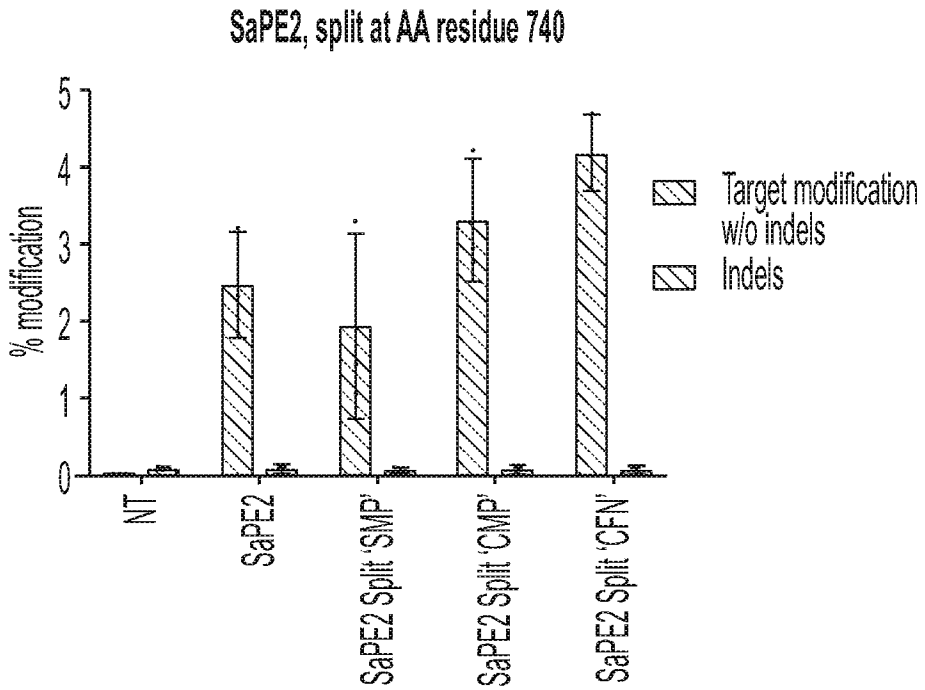


FIG. 68B

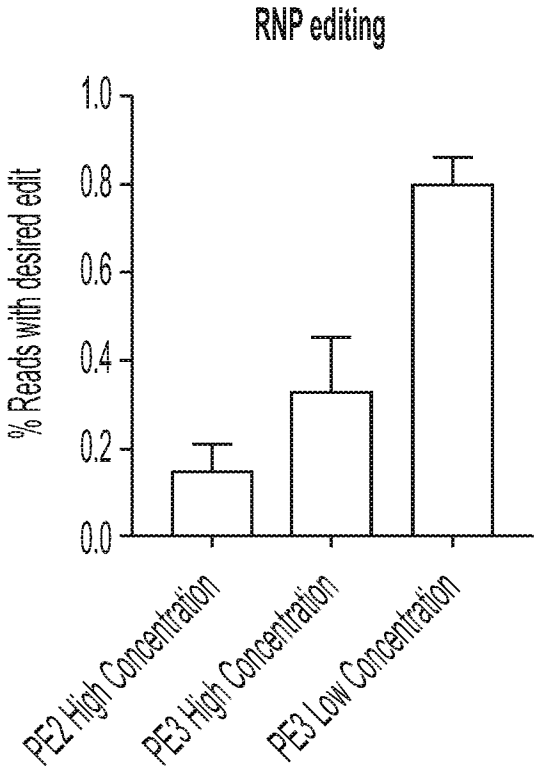

RNP editing
% Reads with desired edit
1.0
0.8
0.6
0.4
0.2
0.0
PE2 High Concentration
PE3 High Concentration
PE3 Low Concentration

FIG. 68C

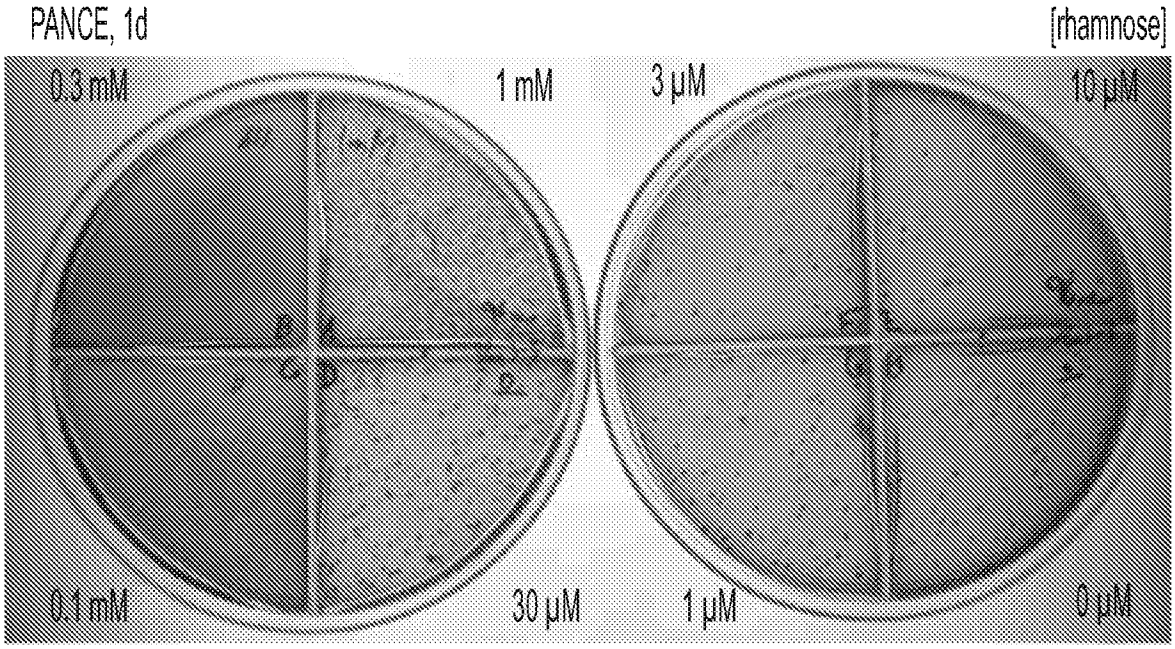


FIG. 69

FIG. 70A

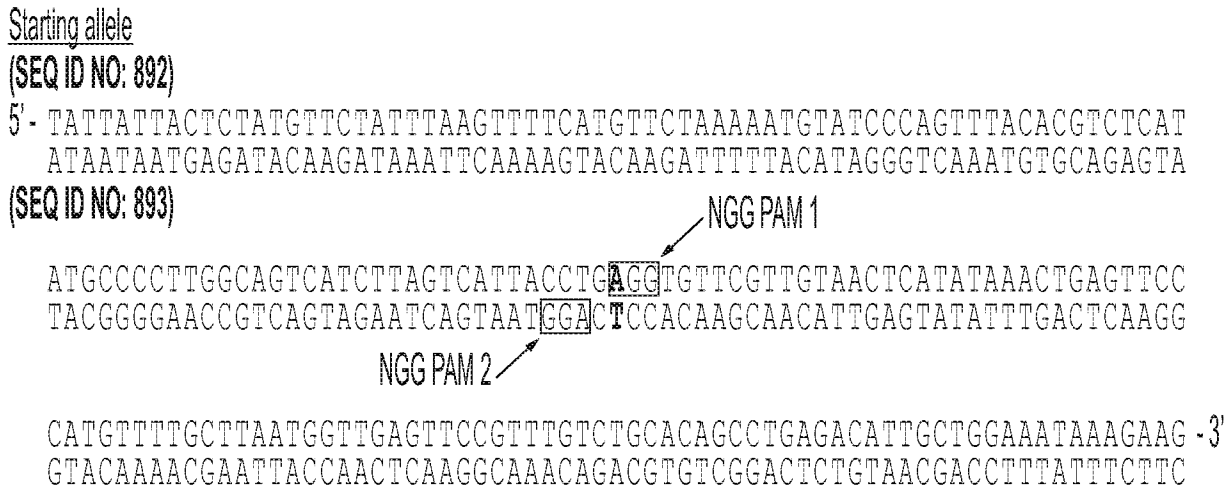


FIG. 70B

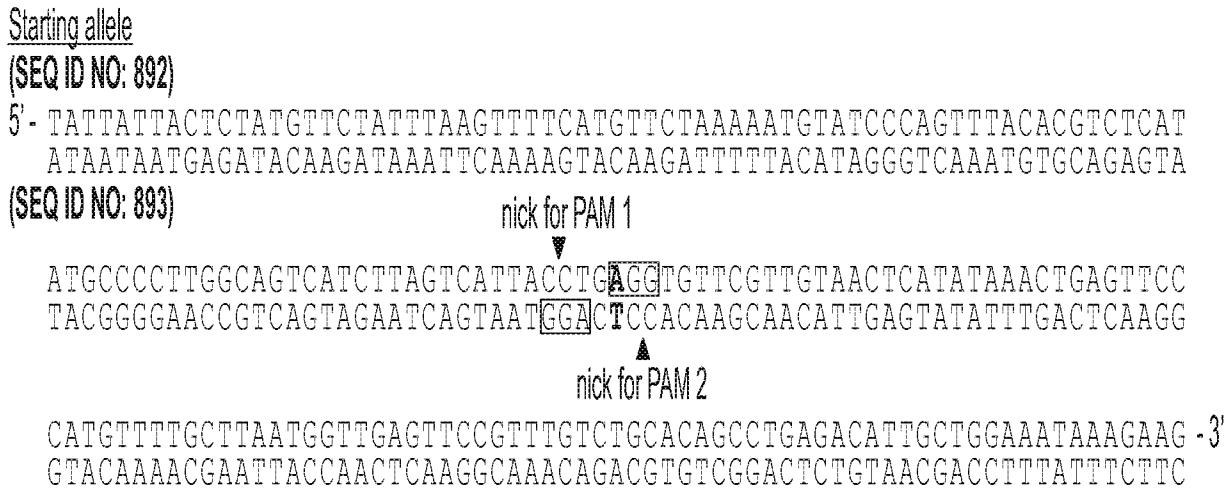


FIG. 70C

FIG. 70D

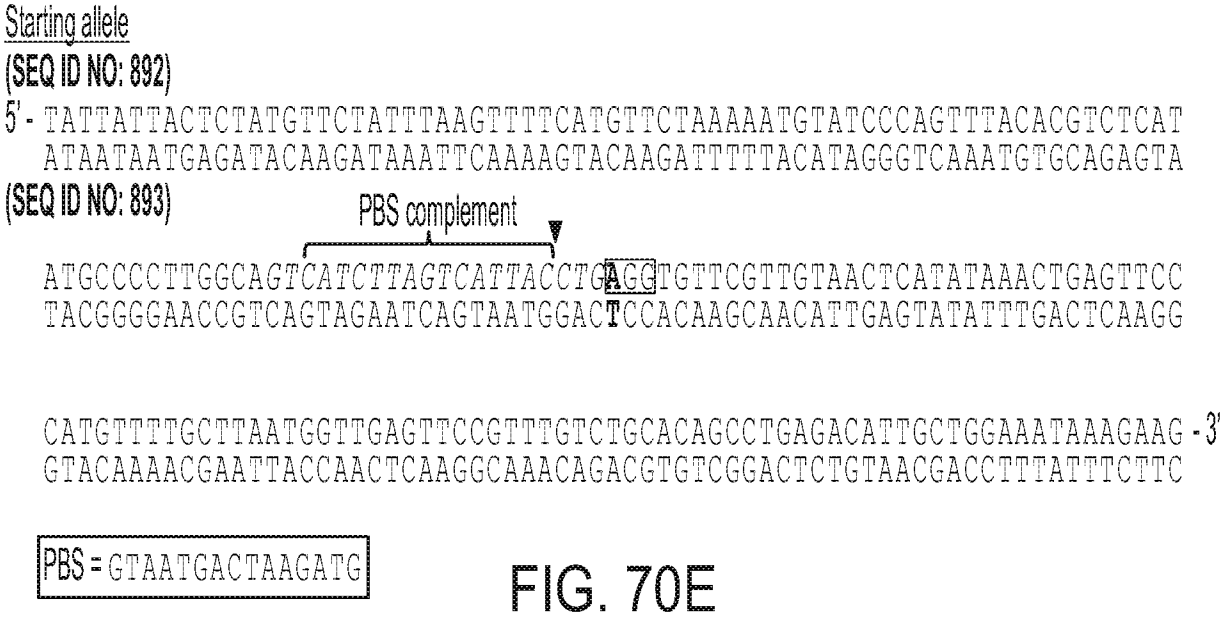


FIG. 70E

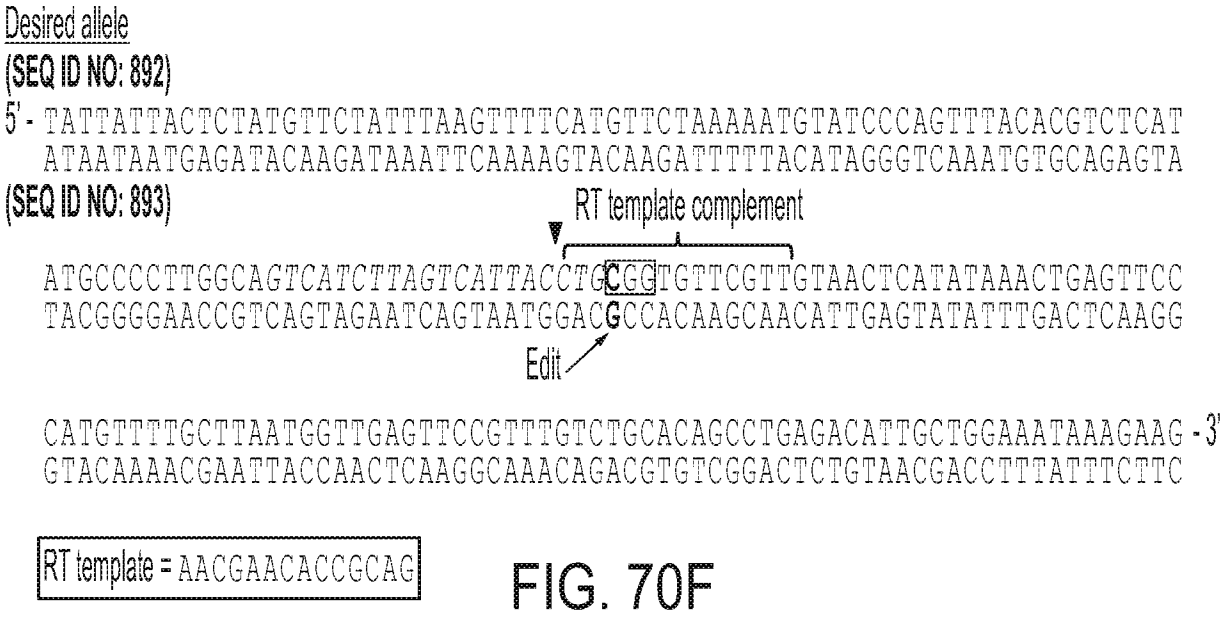


FIG. 70F

FIG. 70G

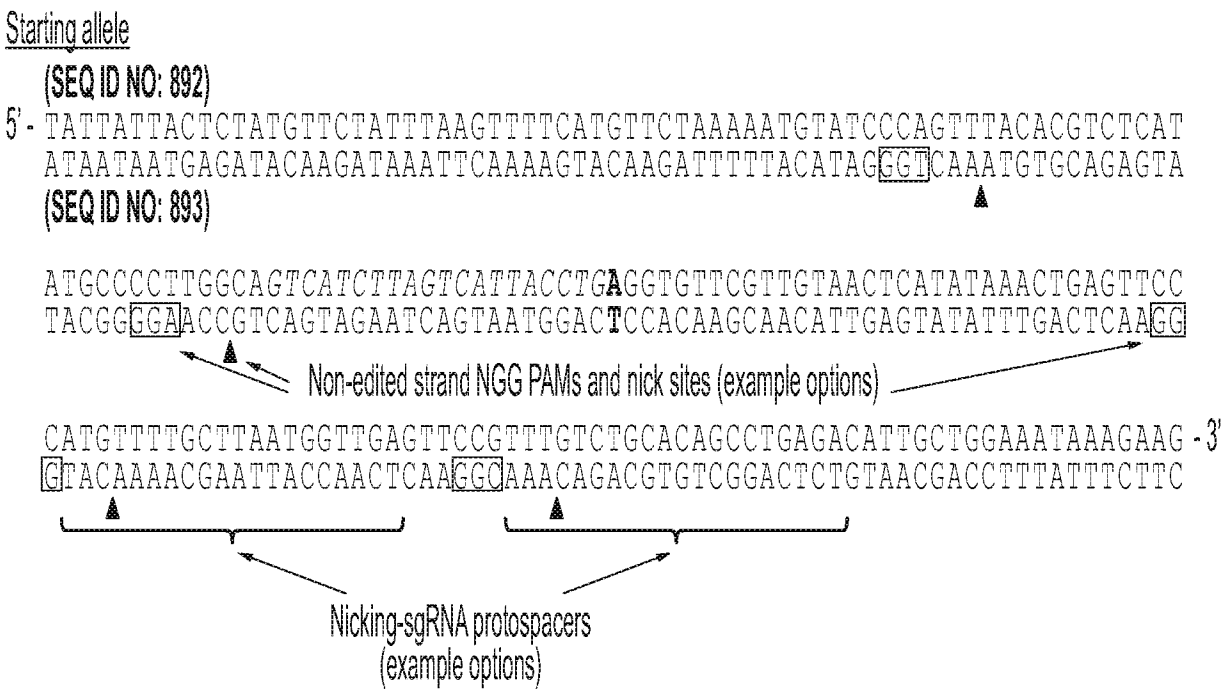


FIG. 70H

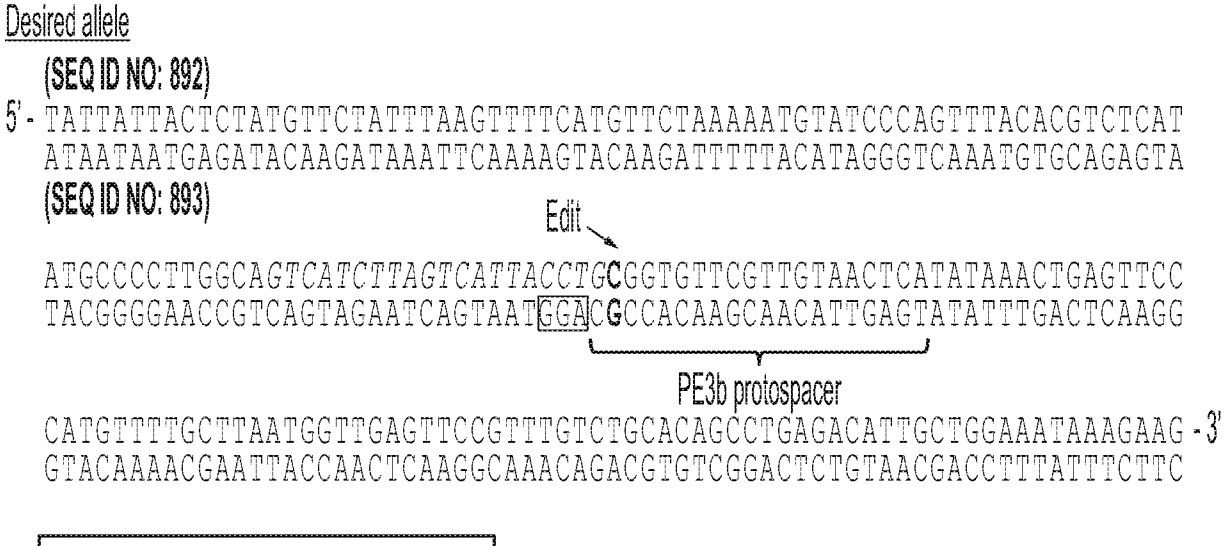


FIG. 70I

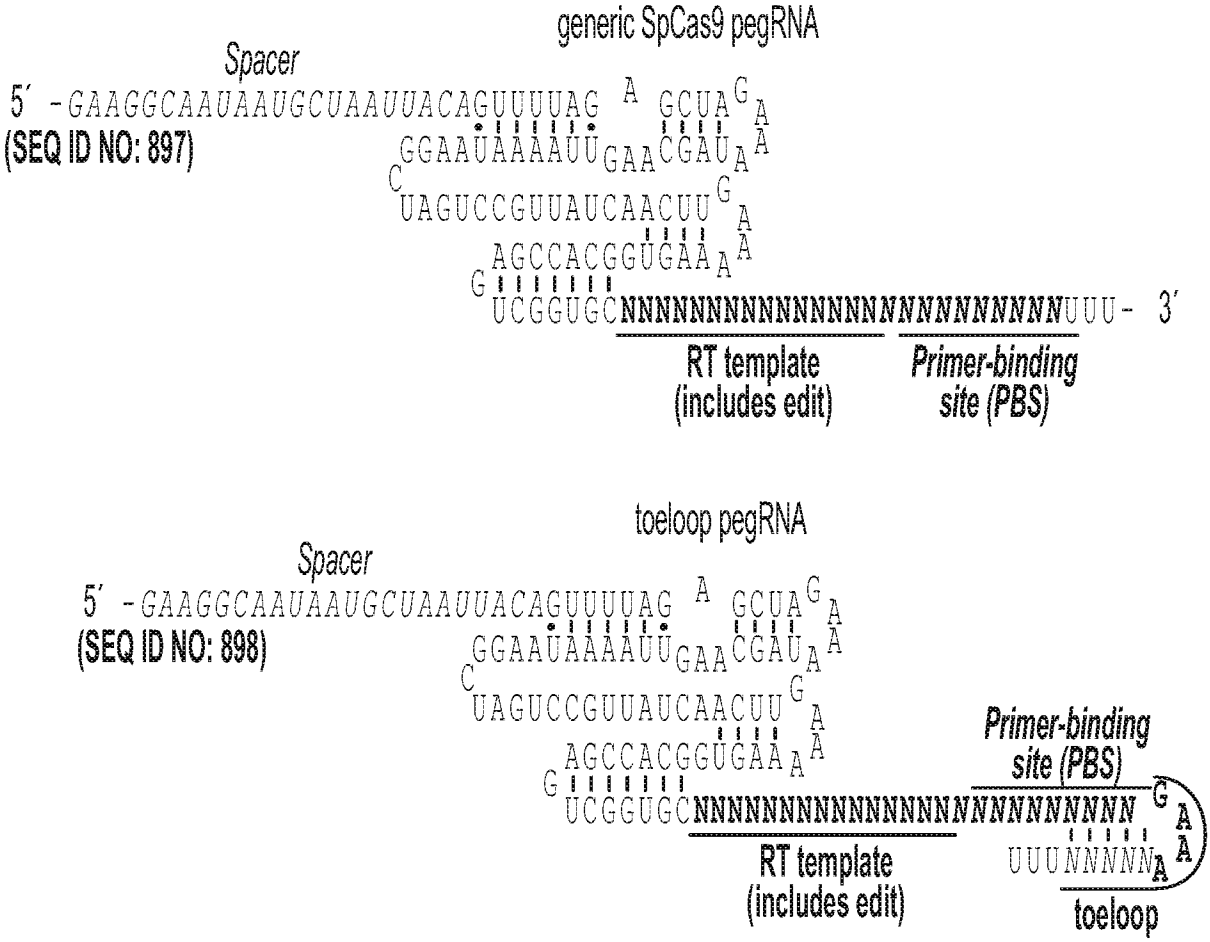


FIG. 71A

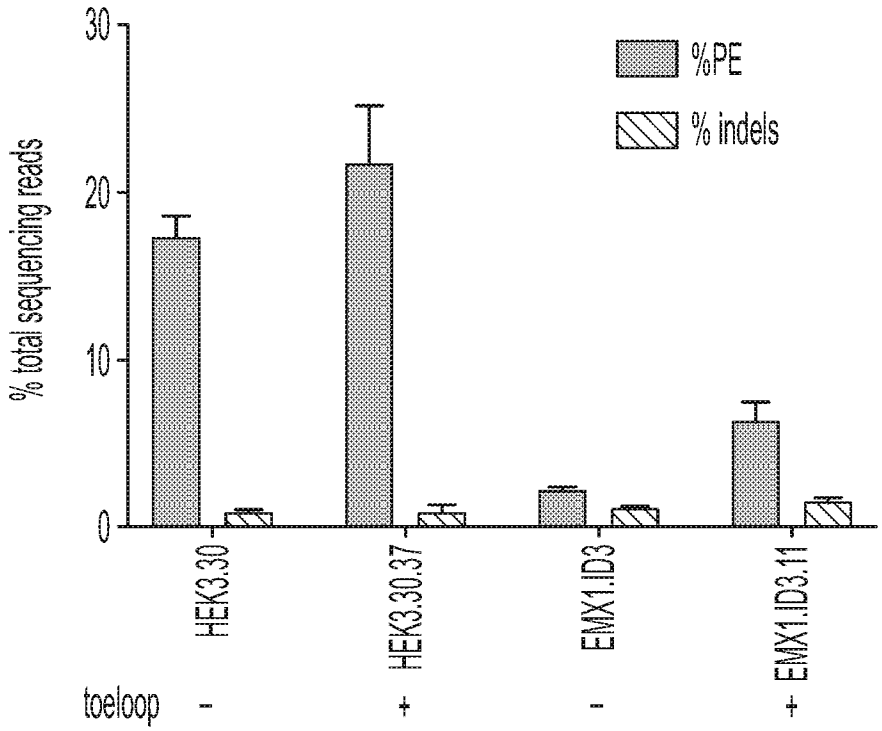


FIG. 71B

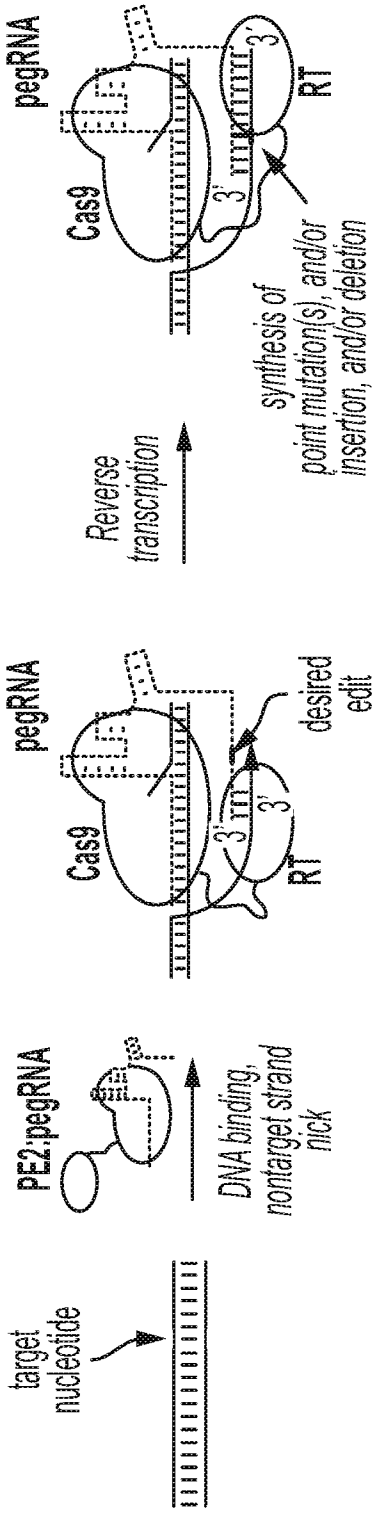


FIG. 72A

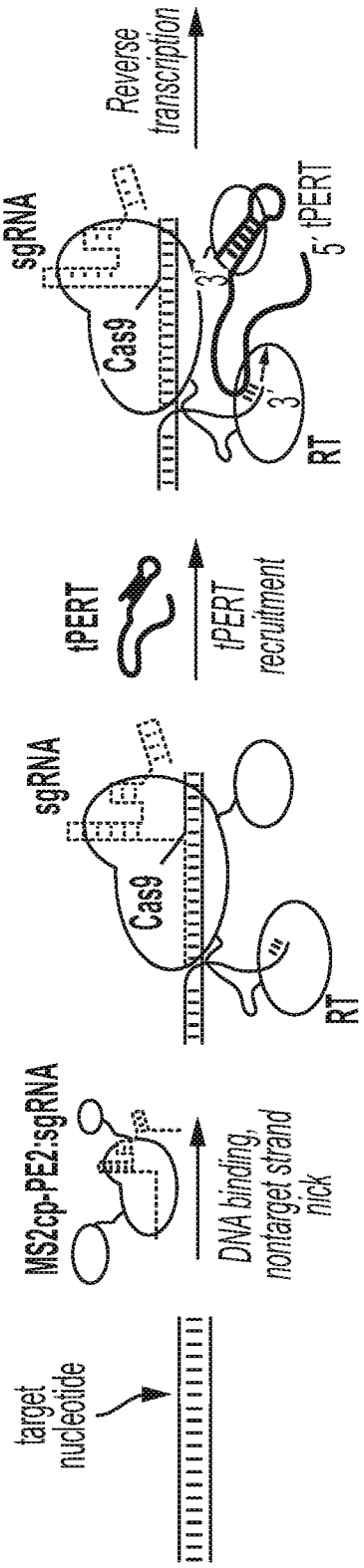


FIG. 72B

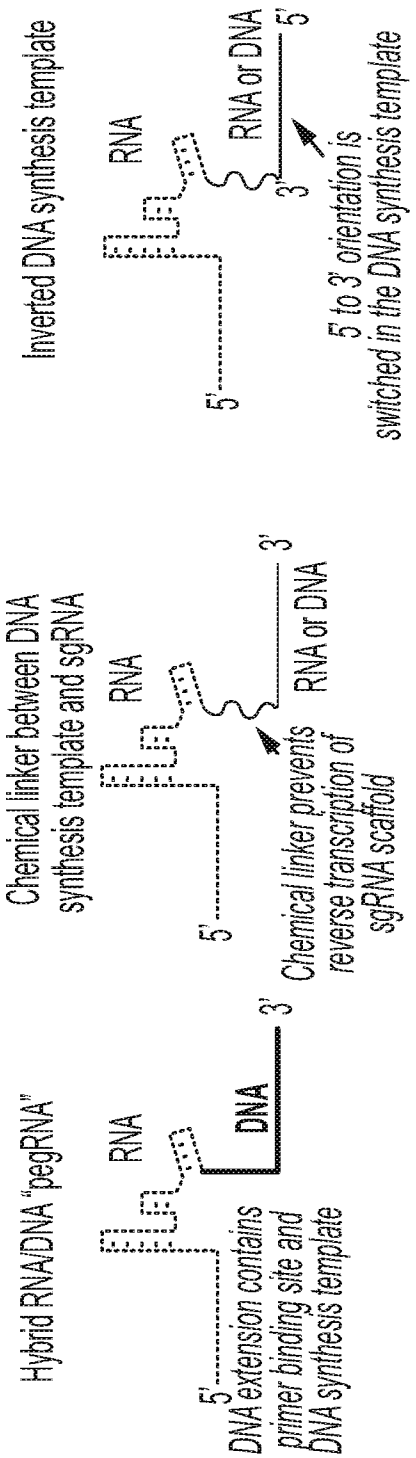
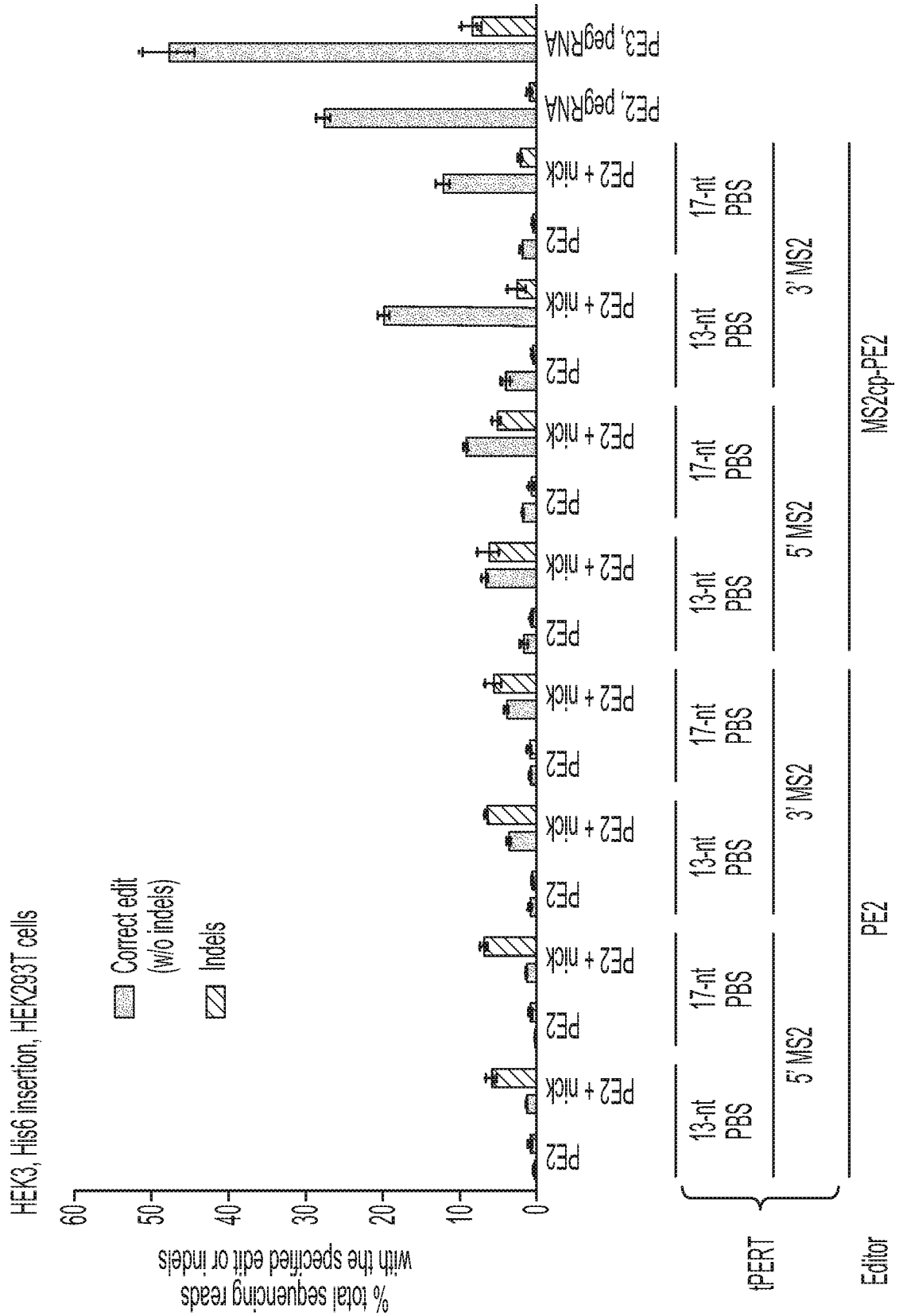

Hybrid RNA/DNA "pegRNA"
RNA
DNA
5'
3'
DNA extension contains primer binding site and DNA synthesis template
DNA-dependent DNA polymerase can be used in place of reverse transcriptase
Chemical linker between DNA synthesis template and sgRNA
RNA
RNA or DNA
5'
3'
Chemical linker prevents reverse transcription of sgRNA scaffold
Inverted DNA synthesis template
RNA
RNA or DNA
5'
3'
5' to 3' orientation is switched in the DNA synthesis template

FIG. 72C


HEK3, His6 insertion, HEK293T cells
Correct edit (w/o indels)
Indels
% total sequencing reads with the specified edit or indels
0
10
20
30
40
50
60
PE2
PE2 + nick
PE2
PE2 + nick
PE2
PE2 + nick

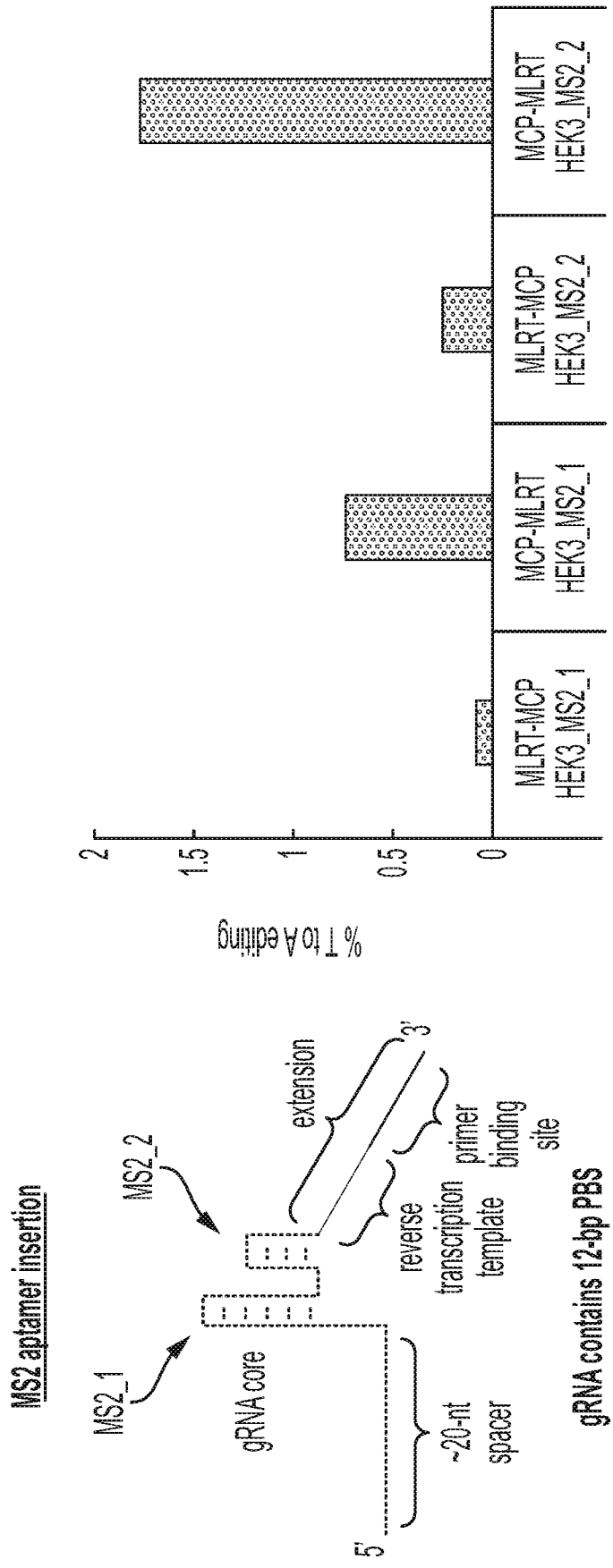

% T to A editing
2
1.5
1
0.5
0
MLRT-MCP HEK3_MS2_1
MCP-MLRT HEK3_MS2_1
MLRT-MCP HEK3_MS2_2
MCP-MLRT HEK3_MS2_2
MS2 aptamer insertion
MS2_1
MS2_2
gRNA core
~20-nt spacer
5'
3'
extension
reverse transcription template
primer binding site
gRNA contains 12-bp PBS

FIG. 74

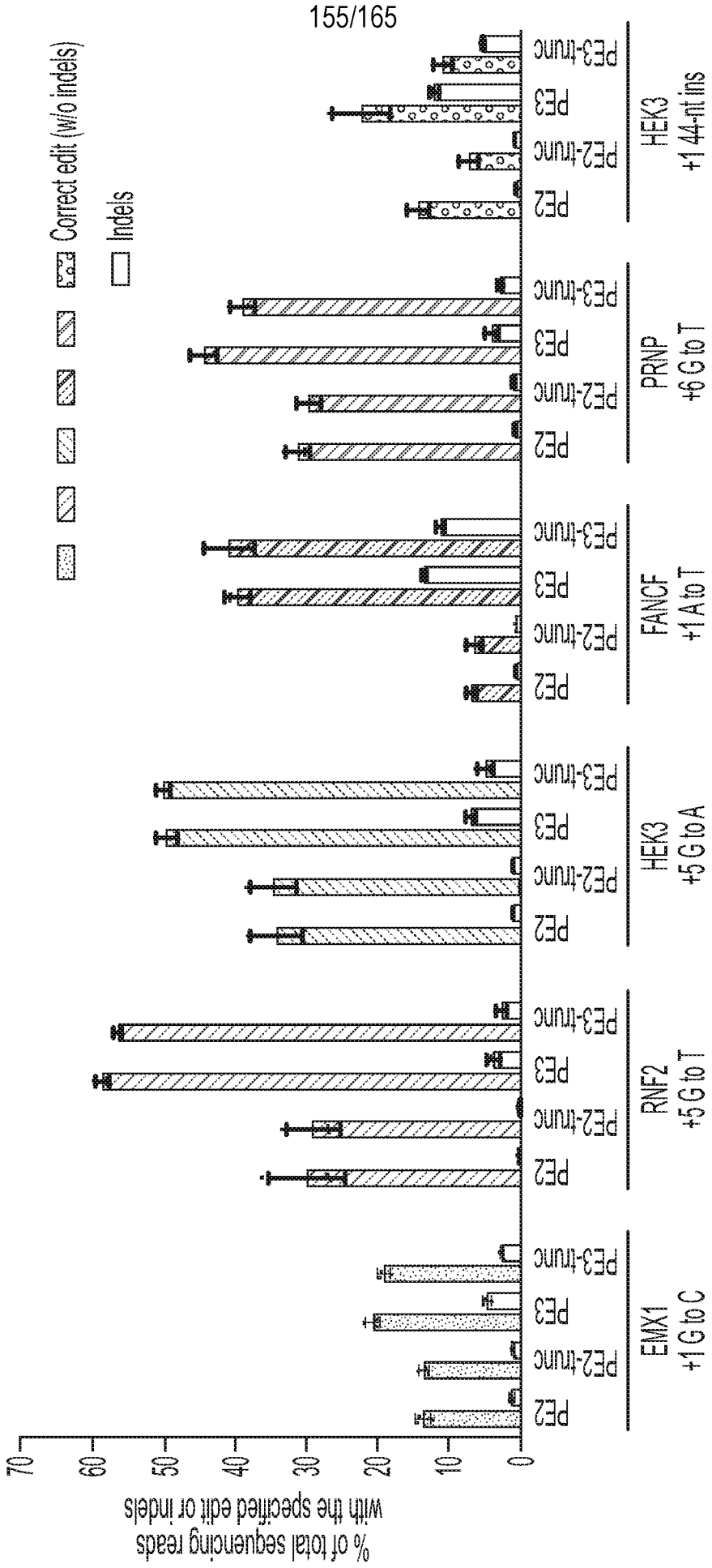

Correct edit (w/o indels)
Indels
% of total sequencing reads with the specified edit or indels
0
10
20
30
40
50
60
70
PE2
PE2-trunc
PE3
PE3-trunc
EMX1 +1 G to C
RNF2 +5 G to T
HEK3 +5 G to A
FANCF +1 A to T
PRNP +6 G to T
HEK3 +1 44-nt ins

FIG. 75

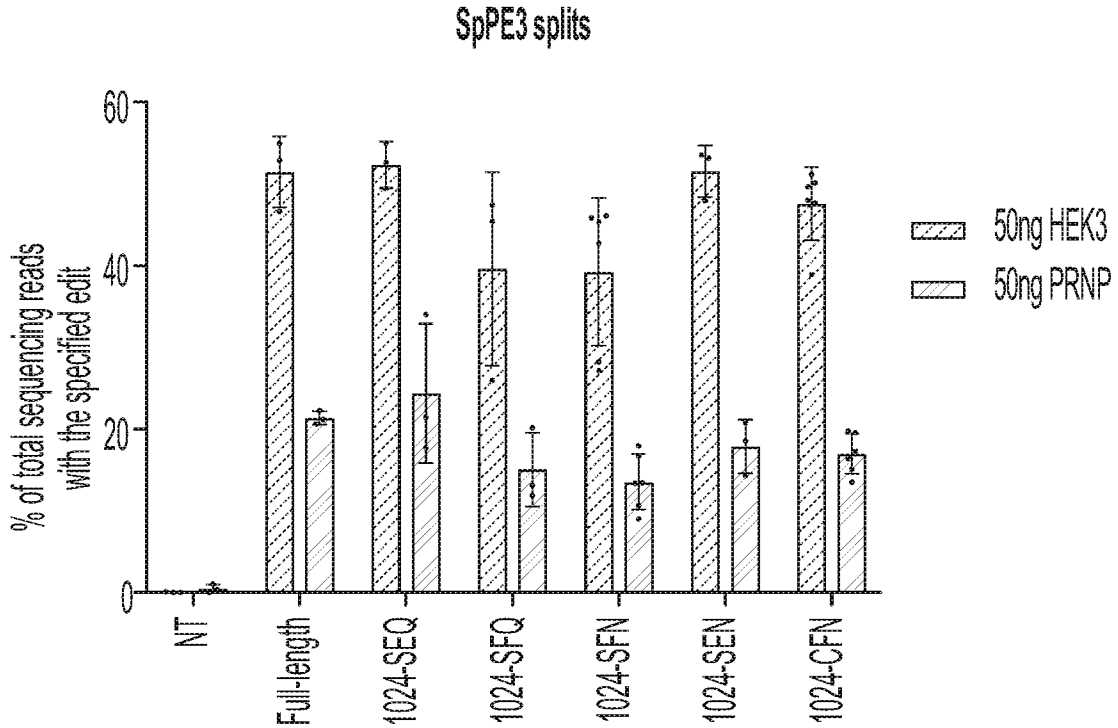

SpPE3 splits
% of total sequencing reads with the specified edit
60
40
20
0
NT
Full-length
1024-SEQ
1024-SFQ
1024-SFN
1024-SEN
1024-CFN
50ng HEK3
50ng PRNP

FIG. 76

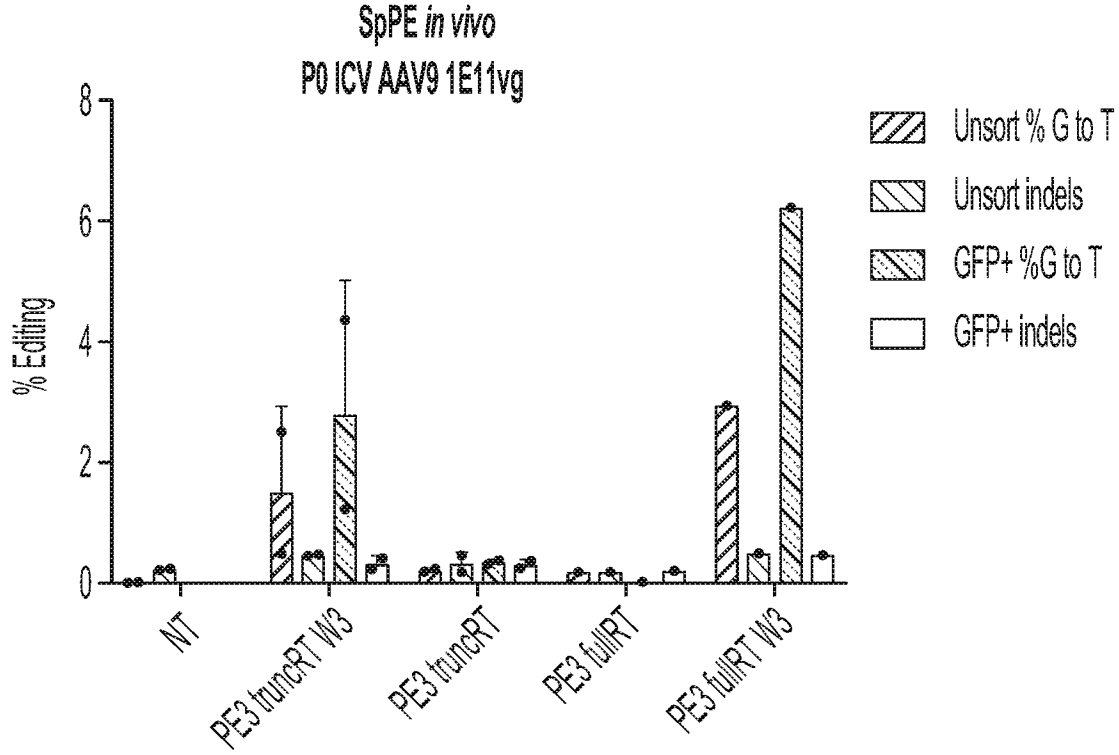

SpPE *in vivo*
P0 ICV AAV9 1E11vg
% Editing
8
6
4
2
0
NT
PE3 truncRT W3
PE3 truncRT
PE3 fullRT
PE3 fullRT W3
Unsort % G to T
Unsort indels
GFP+ %G to T
GFP+ indels

FIG. 77

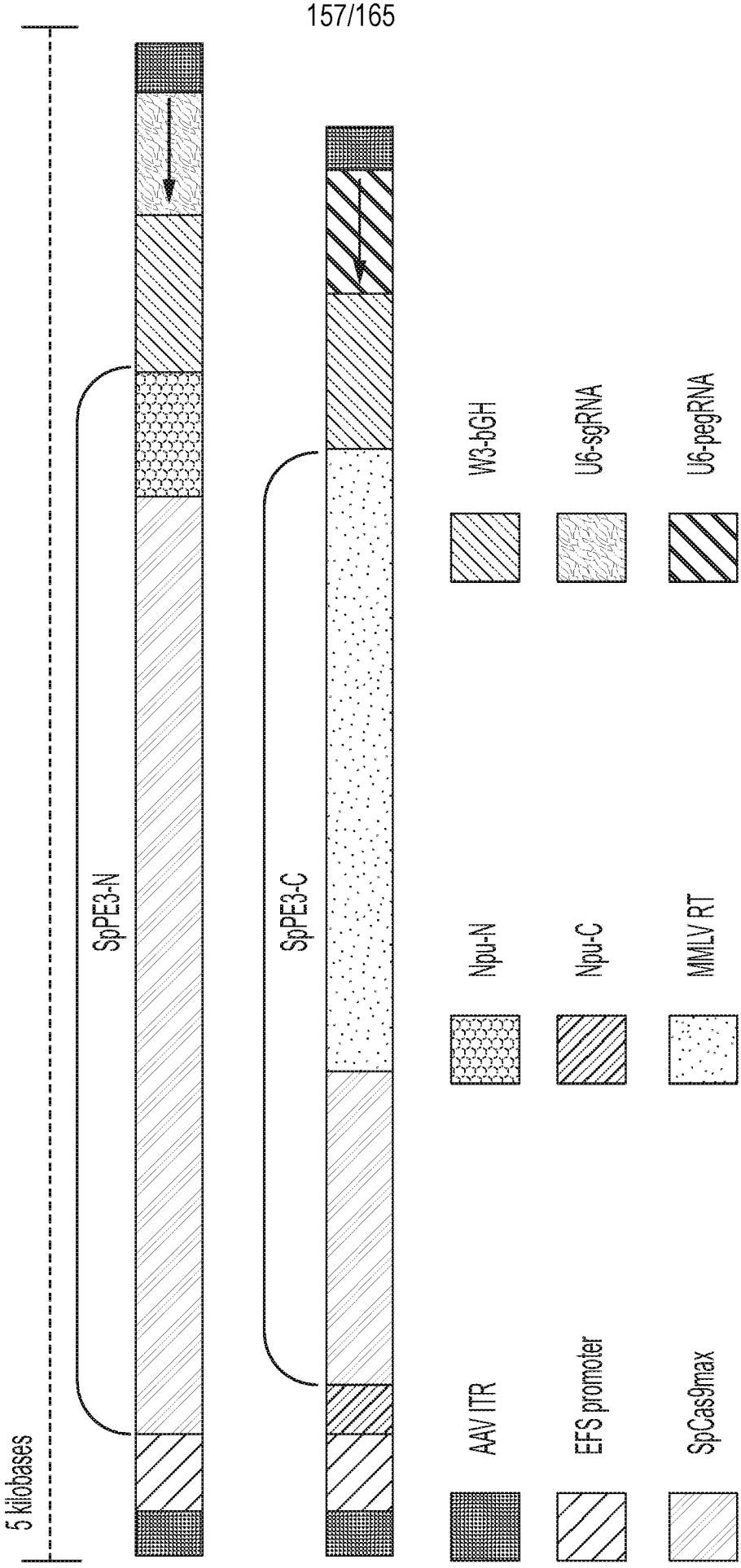

5 kilobases
SpPE3-N
SpPE3-C
AAV ITR
EFS promoter
SpCas9max
Npu-N
Npu-C
MMLV RT
W3-bGH
U6-sgRNA
U6-pegRNA

FIG. 78

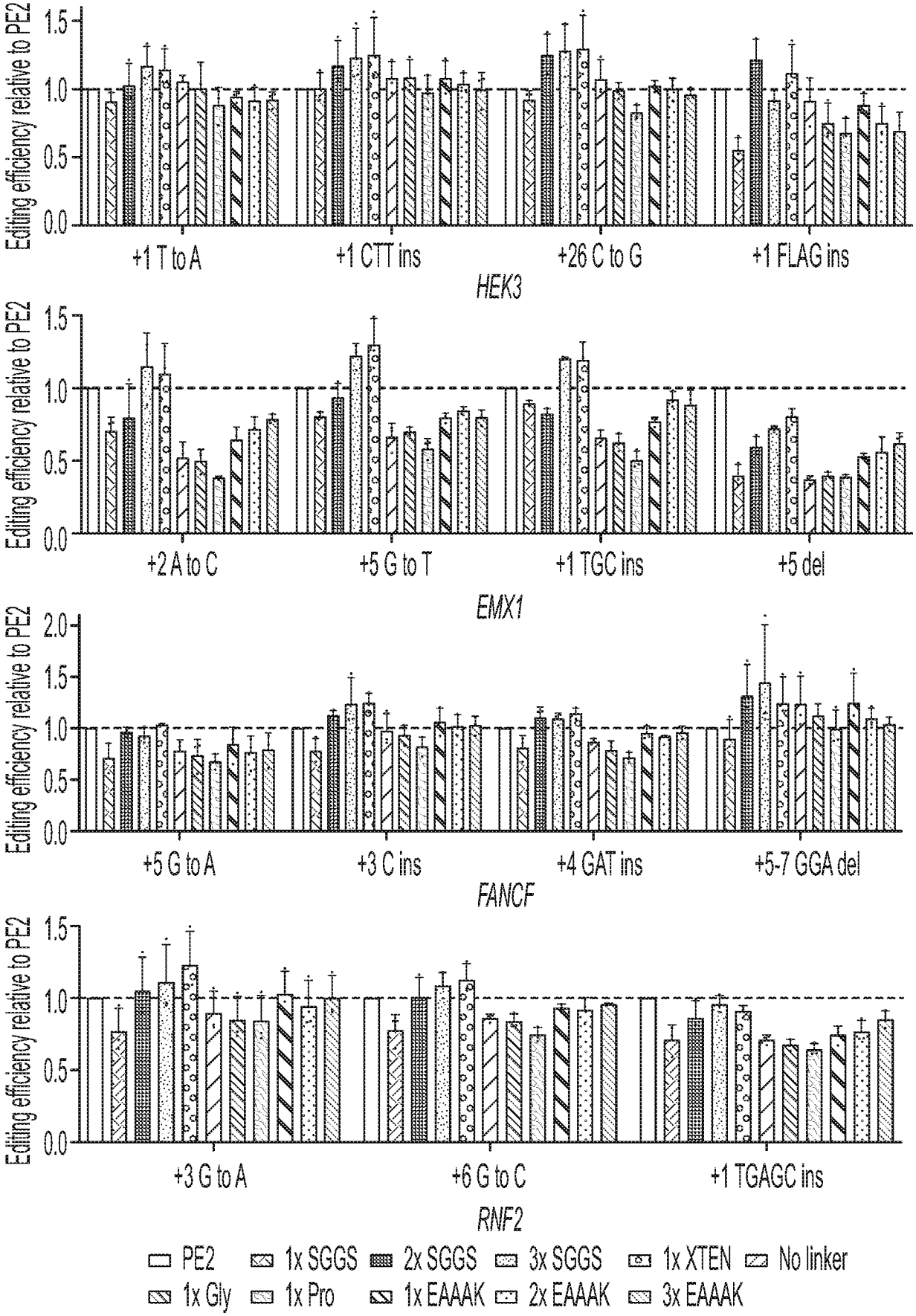

Editing efficiency relative to PE2
1.5
1.0
0.5
0.0
+1 T to A
+1 CTT ins
+26 C to G
+1 FLAG ins
HEK3
+2 A to C
+5 G to T
+1 TGC ins
+5 del
EMX1
2.0
+5 G to A
+3 C ins
+4 GAT ins
+5-7 GGA del
FANCF
+3 G to A
+6 G to C
+1 TGAGC ins
RNF2
PE2
1x SGGS
2x SGGS
3x SGGS
1x XTEN
No linker
1x Gly
1x Pro
1x EAAAK
2x EAAAK
3x EAAAK

FIG. 79

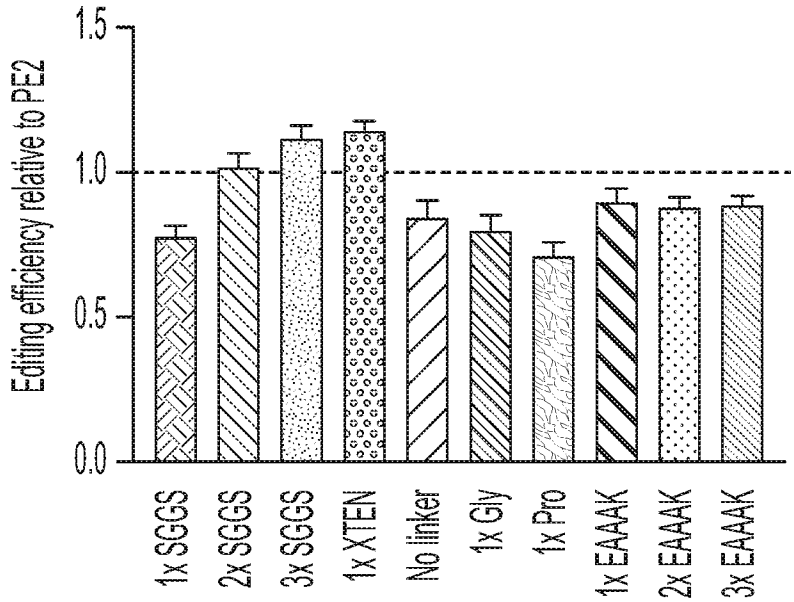

Editing efficiency relative to PE2
1.5
1.0
0.5
0.0
1x SGGS
2x SGGS
3x SGGS
1x XTEN
No linker
1x Gly
1x Pro
1x EAAAK
2x EAAAK
3x EAAAK

FIG. 80

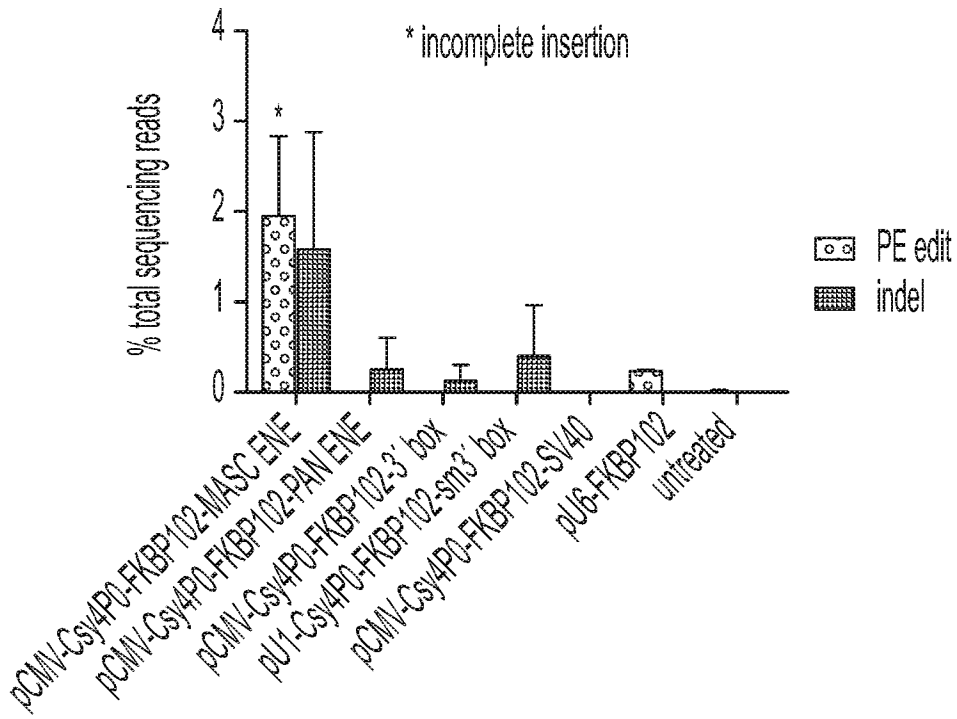

* incomplete insertion
% total sequencing reads
4
3
2
1
0
*
PE edit
indel
pCMV-Csy4P0-FKBP102-MASC ENE
pCMV-Csy4P0-FKBP102-PAN ENE
pCMV-Csy4P0-FKBP102-3' box
pU1-Csy4P0-FKBP102-sm3' box
pCMV-Csy4P0-FKBP102-SV40
pU6-FKBP102
untreated

FIG. 81

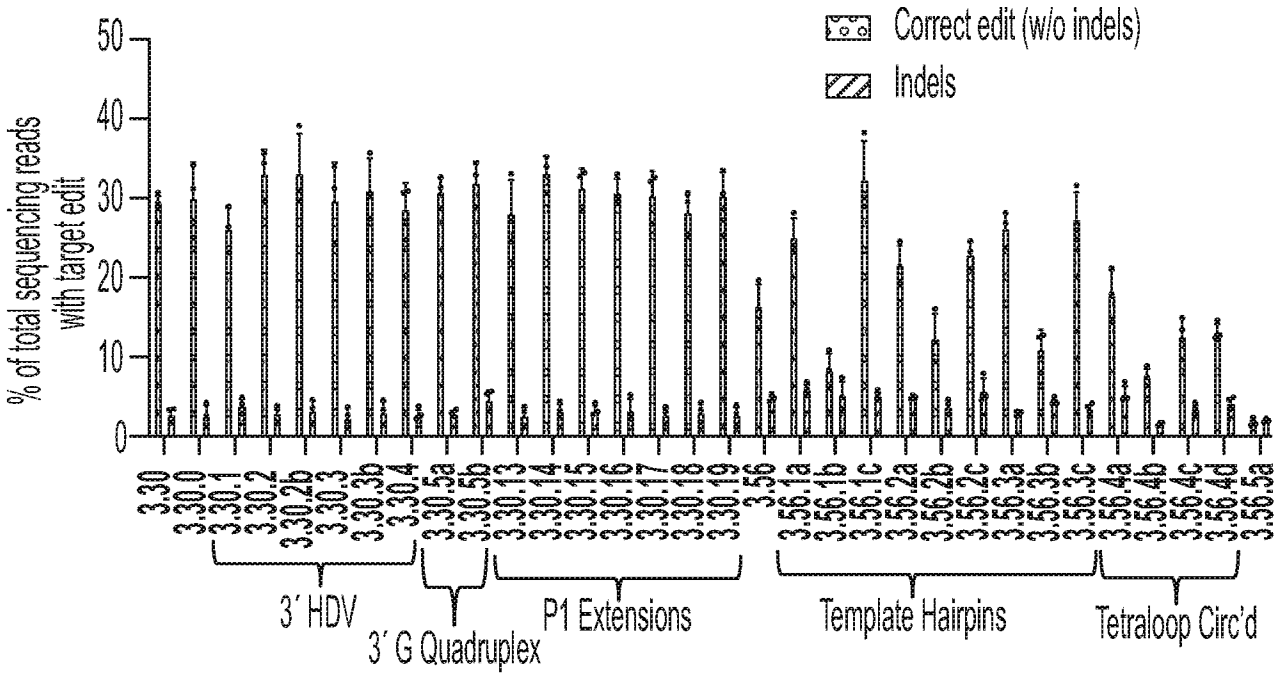

Correct edit (w/o indels)
Indels
% of total sequencing reads with target edit
50
40
30
20
10
0
3.30
3.30.0
3.30.1
3.30.2
3.30.2b
3.30.3
3.30.3b
3.30.4
3.30.5a
3.30.5b
3.30.13
3.30.14
3.30.15
3.30.16
3.30.17
3.30.18
3.30.19
3.56
3.56.1a
3.56.1b
3.56.1c
3.56.2a
3.56.2b
3.56.2c
3.56.3a
3.56.3b
3.56.3c
3.56.4a
3.56.4b
3.56.4c
3.56.4d
3.56.5a
3´ HDV
3´ G Quadruplex
P1 Extensions
Template Hairpins
Tetraloop Circ'd

FIG. 82

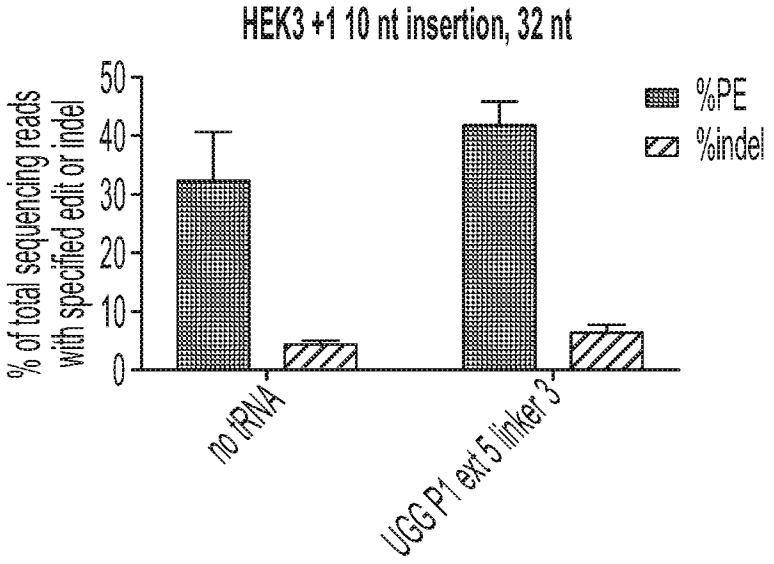

HEK3 +1 10 nt insertion, 32 nt
% of total sequencing reads with specified edit or indel
50
40
30
20
10
0
%PE
%indel
no tRNA
UGG P1 ext 5 linker 3'

FIG. 83

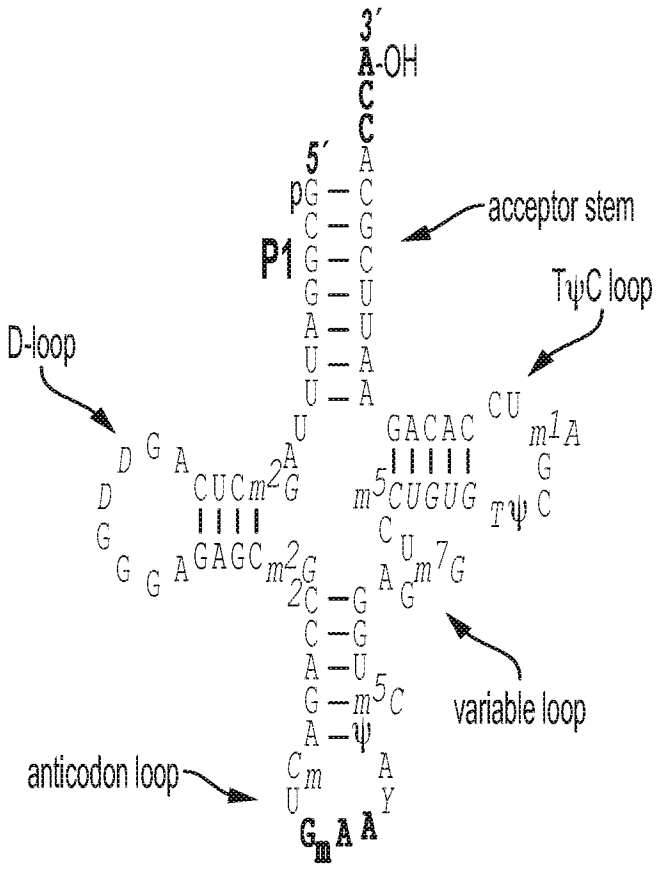


FIG. 84A

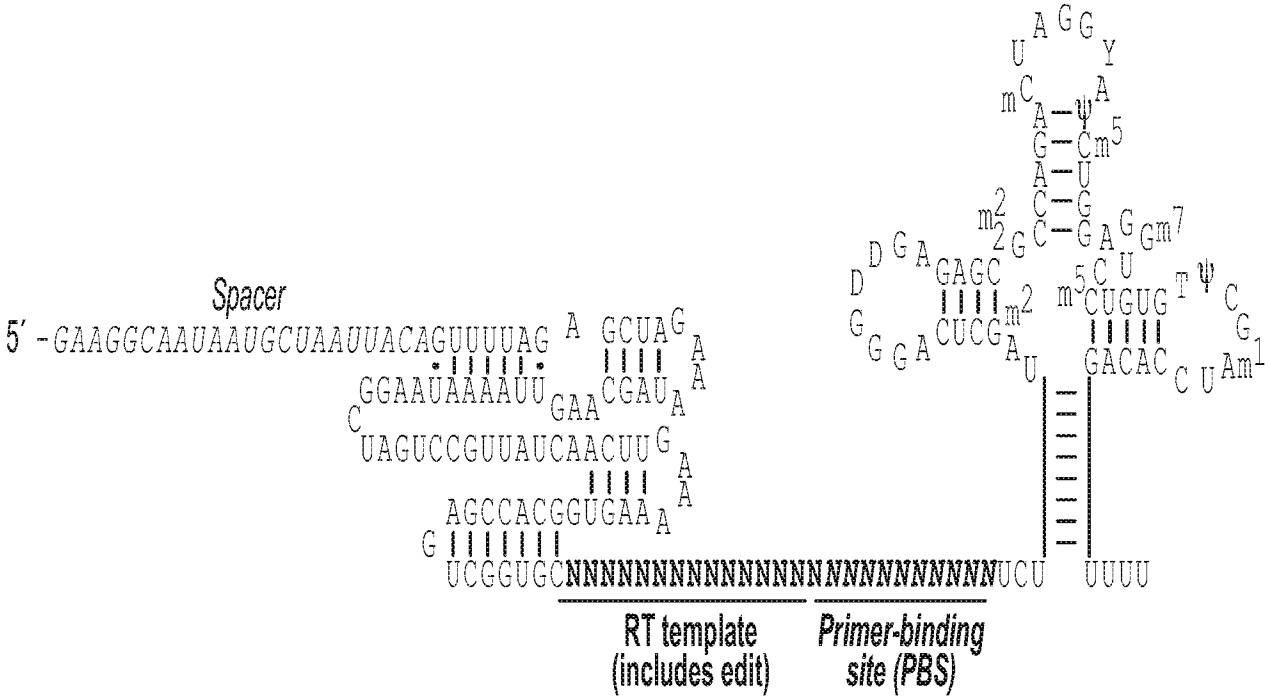


FIG. 84B

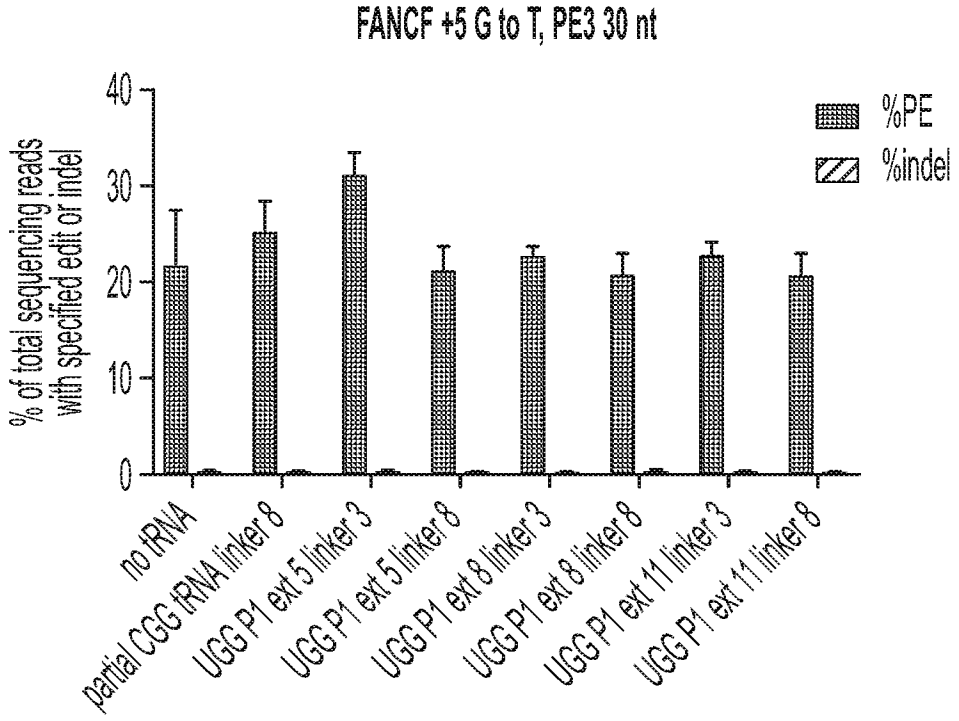

FANCF +5 G to T, PE3 30 nt
% of total sequencing reads with specified edit or indel
40
30
20
10
0
%PE
%indel
no tRNA
partial CGG tRNA linker 8
UGG P1 ext 5 linker 3
UGG P1 ext 5 linker 8
UGG P1 ext 8 linker 3
UGG P1 ext 8 linker 8
UGG P1 ext 11 linker 3
UGG P1 ext 11 linker 8

FIG. 85

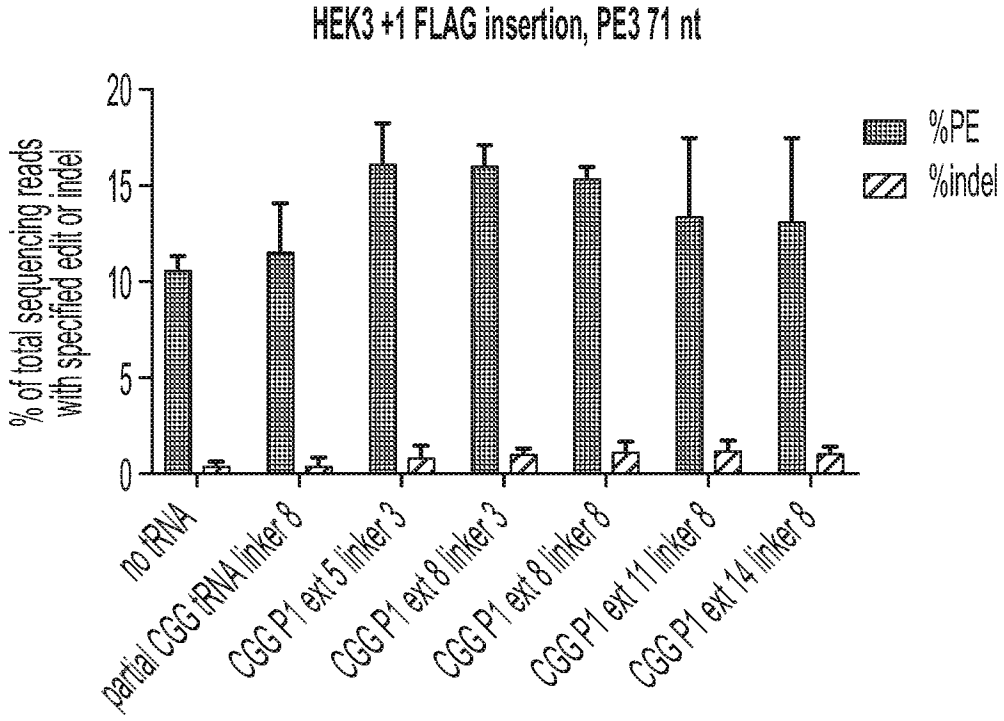

HEK3 +1 FLAG insertion, PE3 71 nt
% of total sequencing reads with specified edit or indel
20
15
10
5
0
%PE
%indel
no tRNA
partial CGG tRNA linker 8
CGG P1 ext 5 linker 3
CGG P1 ext 8 linker 3
CGG P1 ext 8 linker 8
CGG P1 ext 11 linker 8
CGG P1 ext 14 linker 8

FIG. 86

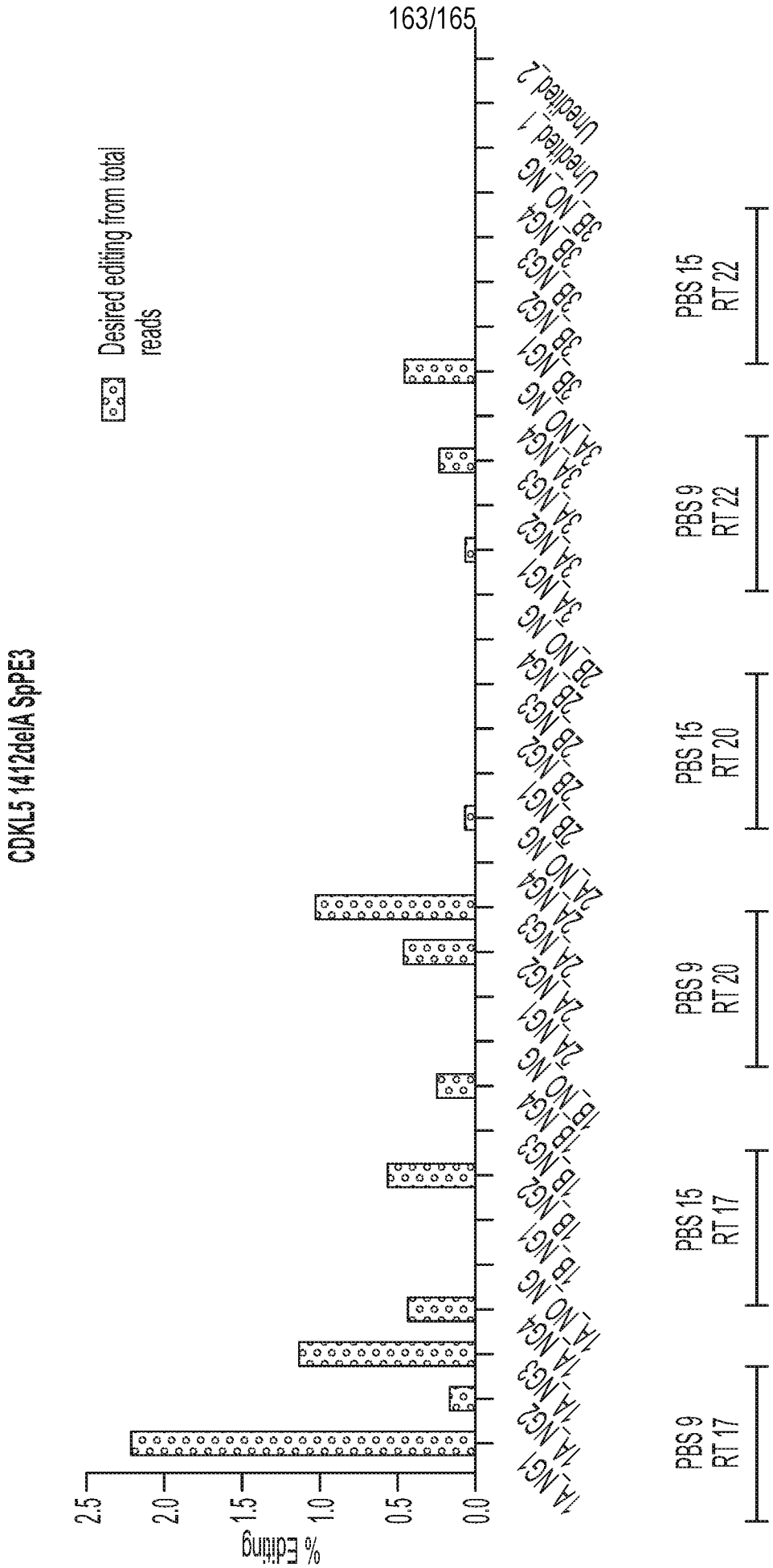

163/165
CDKL5 1412delA SpPE3
Desired editing from total reads
% Editing
2.5
2.0
1.5
1.0
0.5
0.0
1A_NG1
1A_NG2
1A_NG3
1A_NG4
1A_NO_NG
1B_NG1
1B_NG2
1B_NG3
1B_NG4
1B_NO_NG
2A_NG1
2A_NG2
2A_NG3
2A_NG4
2A_NO_NG
2B_NG1
2B_NG2
2B_NG3
2B_NG4
2B_NO_NG
3A_NG1
3A_NG2
3A_NG3
3A_NG4
3A_NO_NG
3B_NG1
3B_NG2
3B_NG3
3B_NG4
3B_NO_NG
Undefined_1
Undefined_2
PBS 9
RT 17
PBS 15
RT 17
PBS 9
RT 20
PBS 15
RT 20
PBS 9
RT 22
PBS 15
RT 22

FIG. 87

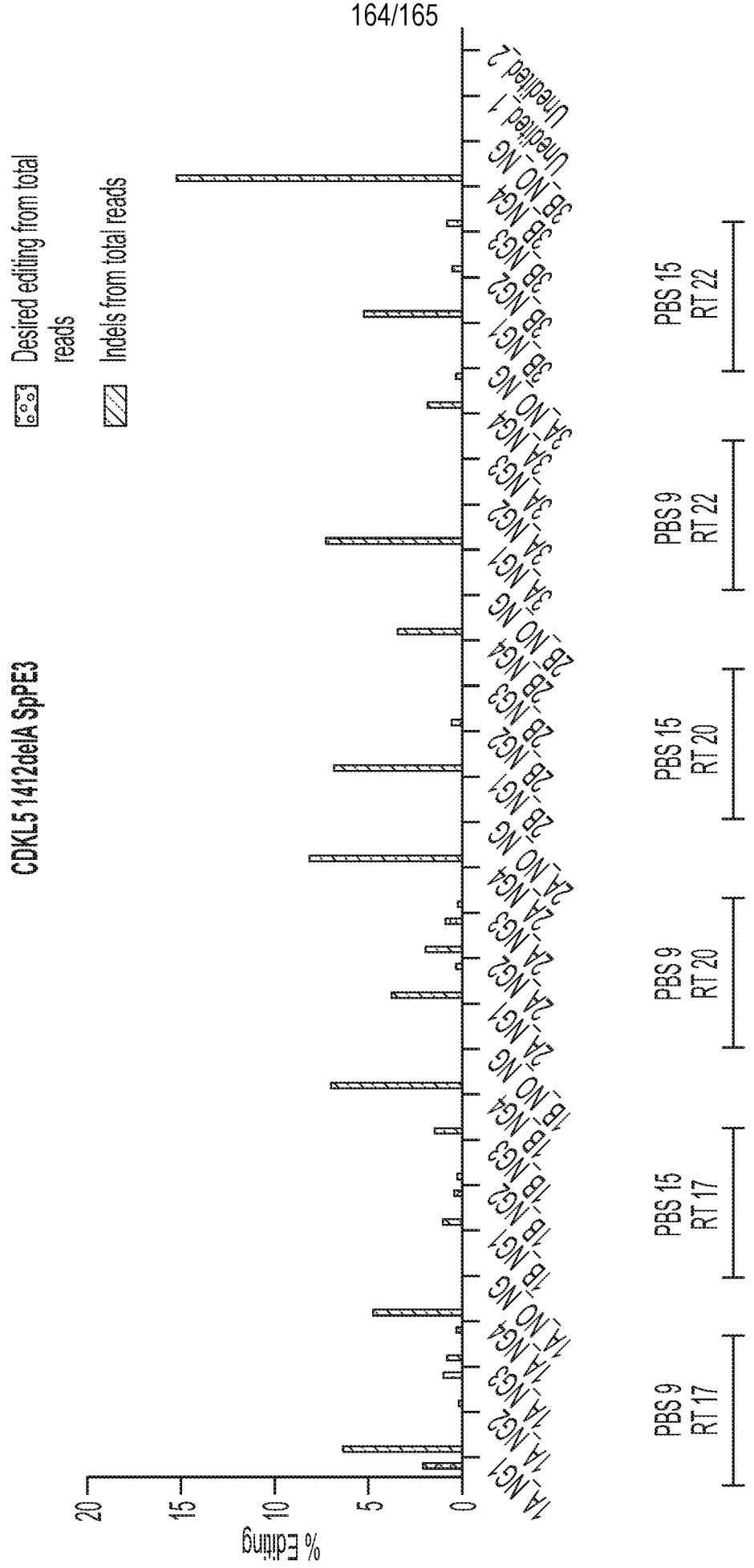

CDKL5 1412delA SpPE3
Desired editing from total reads
Indels from total reads
% Editing
0
5
10
15
20
1A_NG1
1A_1A_NG2
1A_1A_NG3
1A_1A_NG4
1A_NO_NG
1B_1B_NG1
1B_1B_NG2
1B_1B_NG3
1B_1B_NG4
1B_NO_NG
2A_2A_NG1
2A_2A_NG2
2A_2A_NG3
2A_2A_NG4
2A_NO_NG
2B_2B_NG1
2B_2B_NG2
2B_2B_NG3
2B_2B_NG4
2B_NO_NG
3A_3A_NG1
3A_3A_NG2
3A_3A_NG3
3A_3A_NG4
3A_NO_NG
3B_3B_NG1
3B_3B_NG2
3B_3B_NG3
3B_3B_NG4
3B_NO_NG
Unedited_1
Unedited_2
PBS 9
RT 17
PBS 15
RT 17
PBS 9
RT 20
PBS 15
RT 20
PBS 9
RT 22
PBS 15
RT 22

FIG. 88

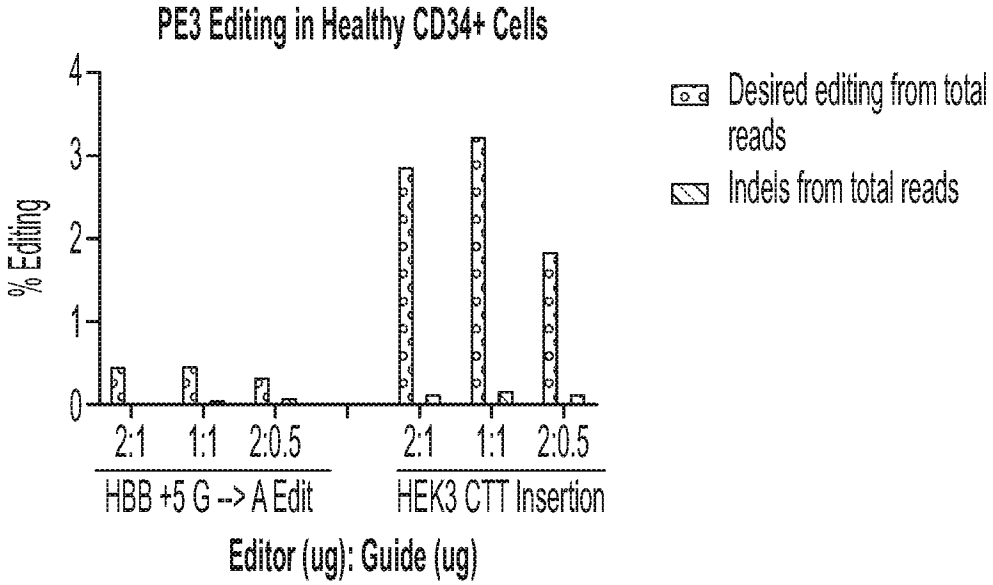

PE3 Editing in Healthy CD34+ Cells
% Editing
0
1
2
3
4
2:1
1:1
2:0.5
2:1
1:1
2:0.5
HBB +5 G --> A Edit
HEK3 CTT Insertion
Editor (ug): Guide (ug)
Desired editing from total reads
Indels from total reads

FIG. 89

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/023727

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11 C12N9/12 C12N9/22 C12N15/10 C12N15/62
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C40B C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/151719 A1 (UNIV FLORIDA [US]) 8 September 2017 (2017-09-08) pages 6,15-19; claims 1-4,21-26; figures 4,7,11; examples 1,6,7	1-5
X	WO 2019/051097 A1 (UNIV CALIFORNIA [US]) 14 March 2019 (2019-03-14) paragraph [0338] - paragraph [0341]; claims 1-4,8,30,32,44-58,77; figure 1	1-5
A	WO 2018/049168 A1 (UNIV LELAND STANFORD JUNIOR [US]) 15 March 2018 (2018-03-15) claims 35,45; figure 1; example 3	1-5
A	WO 2018/165629 A1 (HARVARD COLLEGE [US]) 13 September 2018 (2018-09-13) claim 1; sequence 119	1-5

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 June 2020	12/08/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bucka, Alexander

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HALPERIN SHAKKED O ET AL: "CRISPR-guided DNA polymerases enable diversification of all nucleotides in a tunable window", NATURE,, vol. 560, no. 7717, 1 August 2018 (2018-08-01), pages 248-252, XP036563463, ISSN: 0028-0836, DOI: 10.1038/S41586-018-0384-8 [retrieved on 2018-08-01] figure 1 -----	1-5
X,P	ANZALONE ANDREW V ET AL: "Search-and-replace genome editing without double-strand breaks or donor DNA", NATURE,, vol. 576, no. 7785, 21 October 2019 (2019-10-21), pages 149-157, XP036953141, ISSN: 0028-0836, DOI: 10.1038/S41586-019-1711-4 [retrieved on 2019-10-21] the whole document -----	1-5

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/023727

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/023727

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-5

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5

A method for mutagenizing a DNA molecule at a target locus by prime editing, the method comprising: (a) contacting the DNA molecule at the target locus with a (i) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and an error-prone polymer (e.g., error-prone reverse transcriptase), and (ii) a guide RNA comprising an edit template comprising a desired nucleotide change; thereby polymerizing a single stranded DNA templated from the edit template; and incorporating the single stranded DNA into the DNA molecule at the target locus through a DNA repair and/or replication process.

---

2. claims: 6-11

A method of installing an immunoepitope in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety;
thereby polymerizing a single strand DNA sequence encoding the immunoepitope; thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;
wherein the method produces a recombinant target nucleotide sequence that encodes a fusion protein comprising the protein of interest and the immunoepitope.

---

3. claims: 12-22

A method of installing a small molecule dimerization domain in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase, and (ii) a PEgRNA comprising an edit template encoding the small molecule dimerization domain;
thereby polymerizing a single strand DNA sequence encoding the immunoepitope;
thereby incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process;
wherein the method produces a modified target nucleotide sequence that encodes a fusion protein comprising the

protein of interest and the small molecule dimerization domain.

---

4. claims: 23-32

A method of installing a peptide tag or epitope onto a protein using prime editing, comprising: contacting a target nucleotide sequence encoding the protein with a prime editor construct configured to insert therein a second nucleotide sequence encoding the peptide tag to result in a recombinant nucleotide sequence, such that the peptide tag and the protein are expressed from the recombinant nucleotide sequence as a fusion protein.

---

5. claims: 33, 34

A method of installing or deleting a functional moiety in a protein of interest encoded by a target nucleotide sequence by prime editing, the method comprising: (a) contacting the target nucleotide sequence with a (i) prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a polymerase and (ii) a PEgRNA comprising an edit template encoding the functional moiety or deletion of same; (b) polymerizing a single strand DNA sequence encoding the functional moiety or deletion of same; and (c) incorporating the single strand DNA sequence in place of a corresponding endogenous strand at the target nucleotide sequence through a DNA repair and/or replication process, wherein the method produces a recombinant target nucleotide sequence that encodes a modified protein comprising the protein of interest and the functional moiety or the removal of same, wherein the functional moiety alters a modification state or localization state of the protein.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/023727

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017151719 A1	08-09-2017	US 2019055543 A1	21-02-2019
		WO 2017151719 A1	08-09-2017
WO 2019051097 A1	14-03-2019	US 2020248155 A1	06-08-2020
		WO 2019051097 A1	14-03-2019
WO 2018049168 A1	15-03-2018	EP 3510151 A1	17-07-2019
		US 2019330619 A1	31-10-2019
		WO 2018049168 A1	15-03-2018
WO 2018165629 A1	13-09-2018	CN 110914310 A	24-03-2020
		EP 3592777 A1	15-01-2020
		JP 2020510439 A	09-04-2020
		KR 20190127797 A	13-11-2019
		WO 2018165629 A1	13-09-2018