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(54) Title: RNA-GUIDED EFFECTOR PROTEINS AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides an RNA-guided effector polypeptide having a length that is less than Streptococcus pyogenes Cas9, and that retains the ability, when complexed with a guide RNA, to bind to a target nucleic acid. The present disclosure provides a fusion polypeptide comprising: i) an RNA-guided effector polypeptide of the present disclosure; and ii) a fusion partner. The present disclosure further provides nucleic acids encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. Methods of using an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure are also provided.



RNA-GUIDED EFFECTOR PROTEINS AND METHODS OF USE THEREOF**CROSS-REFERENCE**

- [0001]** This application claims the benefit of U.S. Provisional Patent Application No. 62/690,282, filed June 26, 2018, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

- [0002]** This invention was made with government support under EB018658 awarded by the National Institutes of Health. The government has certain rights in the invention

INCORPORATION BY REFERENCE OF SEQUENCE LISTING PROVIDED AS A TEXT FILE

- [0003]** A Sequence Listing is provided herewith as a text file, "BERK-383WO_SEQ_LISTING_ST25.txt" created on June 18, 2019 and having a size of 7,871 KB. The contents of the text file are incorporated by reference herein in their entirety.

INTRODUCTION

- [0004]** Bacterial adaptive immune systems employ CRISPRs (clustered regularly interspaced short palindromic repeats) and CRISPR-associated (Cas) proteins for RNA-guided nucleic acid cleavage. The CRISPR-Cas systems thereby confer adaptive immunity in bacteria and archaea via RNA-guided nucleic acid interference. To provide anti-viral immunity, processed CRISPR array transcripts (crRNAs) assemble with Cas protein-containing surveillance complexes that recognize nucleic acids bearing sequence complementarity to the virus derived segment of the crRNAs, known as the spacer.
- [0005]** Class 2 CRISPR-Cas systems are streamlined versions in which a single Cas protein (an effector protein, e.g., a type V Cas effector protein such as Cpf1) bound to RNA is responsible for binding to and cleavage of a targeted sequence. The programmable nature of these minimal systems has facilitated their use as a versatile technology that continues to revolutionize the field of genome manipulation.
- [0006]** The physical size of CRISPR-Cas9 proteins can complicate their delivery. There is a need in the art for CRISPR-Cas9 proteins that are smaller than those currently in use.

SUMMARY

[0007] The present disclosure provides an RNA-guided effector polypeptide having a length that is less than *Streptococcus pyogenes* Cas9, and that retains the ability, when complexed with a guide RNA, to bind to a target nucleic acid. The present disclosure provides a fusion polypeptide comprising: i) an RNA-guided effector polypeptide of the present disclosure; and ii) a fusion partner. The present disclosure further provides nucleic acids encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. Methods of using an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0008] **FIG. 1** provide an amino acid sequence of *Streptococcus pyogenes* Cas9 (Spy Cas9).
- [0009] **FIG. 2A-2B** depict start and end sites of deletions of Cas9.
- [0010] **FIG. 3** provides the amino acid sequence of D3CE, an example of an RNA-guided effector polypeptide of the present disclosure.
- [0011] **FIG. 4** provides the amino acid sequence of D4CE, an example of an RNA-guided effector polypeptide of the present disclosure.
- [0012] **FIG. 5A-5B** provide an amino acid sequence alignment of D3CE with Spy Cas9.
- [0013] **FIG. 6A-6B** provide an amino acid sequence alignment of D4CE with Spy Cas9.
- [0014] **FIG. 7A and 7B** provide amino acid sequences of the catalytic domain of a FokI nuclease (**FIG. 7A**) and the “Sharkey” FokI variant (**FIG. 7B**).
- [0015] **FIG. 8** depicts the frequency of highly functional deletion variants across dCas9 sequence. Shown on the graph are the general locations of the A, B, C, and D deletion variants.
- [0016] **FIG. 9** depicts the repression activity of examples of RNA-guided effector polypeptides of the present disclosure, where the RNA-guided effector polypeptides are fused to a transcriptional repressor. Indicated in brackets are the boundaries of the deletion(s).
- [0017] **FIG. 10A and 10B through FIG. 22A and 22B** provide amino acid sequences of examples of RNA-guided effector polypeptides of the present disclosure.
- [0018] **FIG. 23** provides deletion sites of RNA-guided effector polypeptides of the present disclosure.
- [0019] **FIG. 24A-24E** depicts the percentage of mCherry positive mammalian cells in a CRISPR interference assay to assess the function of dCas9 variants with deletion sites in mammalian cells.
- [0020] **FIG. 25** depicts an immunoblotting assay for Flag-tagged MISER-dCas9 or WT-dCas9 KRAB fusion proteins stably expressed in U-251 cells and co-expressing a non-targeting guide (sgNT1).

[0021] FIG. 26 depicts an RNP-based gel shift assay to assess *in vitro* function of variants dCas9, Δ3CE, and Δ4CE.

[0022] FIG. 27 depicts an immunoblotting assay of variants Δ3CE, and Δ4CE that programmably bind to DNA and are functional in human cells.

DEFINITIONS

[0023] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, terms “polynucleotide” and “nucleic acid” encompass single-stranded DNA; double-stranded DNA; multi-stranded DNA; single-stranded RNA; double-stranded RNA; multi-stranded RNA; genomic DNA; cDNA; DNA-RNA hybrids; and a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[0024] The term “oligonucleotide” refers to a polynucleotide of between 4 and 100 nucleotides of single- or double-stranded nucleic acid (e.g., DNA, RNA, or a modified nucleic acid). However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as “oligomers” or “oligos” and can be isolated from genes, transcribed (*in vitro* and/or *in vivo*), or chemically synthesized. The terms “polynucleotide” and “nucleic acid” should be understood to include, as applicable to the embodiments being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

[0025] By “hybridizable” or “complementary” or “substantially complementary” it is meant that a nucleic acid (e.g. RNA, DNA) comprises a sequence of nucleotides that enables it to non-covalently bind, i.e. form Watson-Crick base pairs and/or G/U base pairs, “anneal”, or “hybridize,” to another nucleic acid in a sequence-specific, antiparallel, manner (i.e., a nucleic acid specifically binds to a complementary nucleic acid) under the appropriate *in vitro* and/or *in vivo* conditions of temperature and solution ionic strength. Standard Watson-Crick base-pairing includes: adenine/adenosine (A) pairing with thymine/thymidine (T), A pairing with uracil/uridine (U), and guanine/guanosine (G) pairing with cytosine/cytidine (C). In addition, for hybridization between two RNA molecules (e.g., dsRNA), and for hybridization of a DNA molecule with an RNA molecule (e.g., when a DNA target nucleic acid base pairs with a guide RNA, etc.): G can also base pair with U. For example, G/U base-pairing is partially responsible for the degeneracy (i.e., redundancy) of the genetic code in the context of tRNA anti-codon base-pairing with codons in mRNA. Thus, in the context of this disclosure, a G (e.g., of a protein-

binding segment (e.g., dsRNA duplex) of a guide RNA molecule; of a target nucleic acid (e.g., target DNA) base pairing with a guide RNA) is considered complementary to both a U and to C. For example, when a G/U base-pair can be made at a given nucleotide position of a protein-binding segment (e.g., dsRNA duplex) of a guide RNA molecule, the position is not considered to be non-complementary, but is instead considered to be complementary.

[0026] Hybridization requires that the two nucleic acids contain complementary sequences, although mismatches between bases are possible. The conditions appropriate for hybridization between two nucleic acids depend on the length of the nucleic acids and the degree of complementarity, variables well known in the art. The greater the degree of complementarity between two nucleotide sequences, the greater the value of the melting temperature (T_m) for hybrids of nucleic acids having those sequences. Typically, the length for a hybridizable nucleic acid is 8 nucleotides or more (e.g., 10 nucleotides or more, 12 nucleotides or more, 15 nucleotides or more, 20 nucleotides or more, 22 nucleotides or more, 25 nucleotides or more, or 30 nucleotides or more).

[0027] It is understood that the sequence of a polynucleotide need not be 100% complementary to that of its target nucleic acid to be specifically hybridizable. Moreover, a polynucleotide may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or hairpin structure, a 'bulge', and the like). A polynucleotide can comprise 60% or more, 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, 99.5% or more, or 100% sequence complementarity to a target region within the target nucleic acid sequence to which it will hybridize. For example, an antisense nucleic acid in which 18 of 20 nucleotides of the antisense compound are complementary to a target region, and would therefore specifically hybridize, would represent 90 percent complementarity. The remaining noncomplementary nucleotides may be clustered or interspersed with complementary nucleotides and need not be contiguous to each other or to complementary nucleotides. Percent complementarity between particular stretches of nucleic acid sequences within nucleic acids can be determined using any convenient method. Example methods include BLAST programs (basic local alignment search tools) and PowerBLAST programs (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656) or by using the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison Wis.), e.g., using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489).

[0028] The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded

amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

[0029] "Binding" as used herein (e.g. with reference to an RNA-binding domain of a polypeptide, binding to a target nucleic acid, and the like) refers to a non-covalent interaction between macromolecules (e.g., between a protein and a nucleic acid; between a guide RNA and a target nucleic acid; and the like). While in a state of non-covalent interaction, the macromolecules are said to be "associated" or "interacting" or "binding" (e.g., when a molecule X is said to interact with a molecule Y, it is meant the molecule X binds to molecule Y in a non-covalent manner). Not all components of a binding interaction need be sequence-specific (e.g., contacts with phosphate residues in a DNA backbone), but some portions of a binding interaction may be sequence-specific. Binding interactions are generally characterized by a dissociation constant (K_d) of less than 10^{-6} M, less than 10^{-7} M, less than 10^{-8} M, less than 10^{-9} M, less than 10^{-10} M, less than 10^{-11} M, less than 10^{-12} M, less than 10^{-13} M, less than 10^{-14} M, or less than 10^{-15} M. "Affinity" refers to the strength of binding, increased binding affinity being correlated with a lower K_d .

[0030] By "binding domain" it is meant a protein domain that is able to bind non-covalently to another molecule. A binding domain can bind to, for example, an RNA molecule (an RNA-binding domain) and/or a protein molecule (a protein-binding domain). In the case of a protein having a protein-binding domain, it can in some cases bind to itself (to form homodimers, homotrimers, etc.) and/or it can bind to one or more regions of a different protein or proteins.

[0031] The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of amino acids having amide containing side chains consisting of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; a group of amino acids having acidic side chains consists of glutamate and aspartate; and a group of amino acids having sulfur containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine-glycine, and asparagine-glutamine.

[0032] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences.

Sequence identity can be determined in a number of different ways. To determine sequence identity, sequences can be aligned using various methods and computer programs (e.g., BLAST, T-COFFEE, MUSCLE, MAFFT, Phyre2, etc.), available over the world wide web at sites including [ncbi\(dot\)nlm\(dot\)nili\(dot\)gov/BLAST](http://ncbi(dot)nlm(dot)nili(dot)gov/BLAST), [ebi\(dot\)ac\(dot\)uk/Tools/msa/tcoffee/](http://ebi(dot)ac(dot)uk/Tools/msa/tcoffee/), [ebi\(dot\)ac\(dot\)uk/Tools/msa/muscle/](http://ebi(dot)ac(dot)uk/Tools/msa/muscle/), [mafft\(dot\)cbrc\(dot\)jp/alignment/software/](http://mafft(dot)cbrc(dot)jp/alignment/software/), [www\(dot\)sbg\(dot\)bio\(dot\)ic\(dot\)ac\(dot\)uk/~phyre2/](http://www(dot)sbg(dot)bio(dot)ic(dot)ac(dot)uk/~phyre2/). See, e.g., Altschul et al. (1990), J. Mol. Bioi. 215:403-10.

- [0033]** The terms "DNA regulatory sequences," "control elements," and "regulatory elements," used interchangeably herein, refer to transcriptional and translational control sequences, such as promoters, enhancers, polyadenylation signals, terminators, protein degradation signals, and the like, that provide for and/or regulate transcription of a non-coding sequence (e.g., guide RNA) or a coding sequence (e.g., protein coding) and/or regulate translation of an encoded polypeptide.
- [0034]** As used herein, a "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase and initiating transcription of a downstream (3' direction) coding or non-coding sequence. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Various promoters, including inducible promoters, may be used to drive the various nucleic acids (e.g., vectors) of the present disclosure.
- [0035]** The term "naturally-occurring" or "unmodified" or "wild type" as used herein as applied to a nucleic acid, a polypeptide, a cell, or an organism, refers to a nucleic acid, polypeptide, cell, or organism that is found in nature.
- [0036]** "Recombinant," as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, polymerase chain reaction (PCR) and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. DNA sequences encoding polypeptides can be assembled from cDNA fragments or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system. Genomic DNA comprising the relevant sequences can also be used in the formation of a recombinant gene or transcriptional unit. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where such sequences do not interfere with manipulation or expression of the coding regions, and may indeed act to modulate production of a desired product by various mechanisms (see "DNA regulatory sequences", below). Alternatively, DNA sequences encoding RNA (e.g., guide RNA) that is not translated may also be considered recombinant. Thus, e.g., the term "recombinant" nucleic acid refers to one which is not naturally occurring, e.g., is made by the artificial combination of two otherwise separated segments of

sequence through human intervention. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a codon encoding the same amino acid, a conservative amino acid, or a non-conservative amino acid. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. When a recombinant polynucleotide encodes a polypeptide, the sequence of the encoded polypeptide can be naturally occurring ("wild type") or can be a variant (e.g., a mutant) of the naturally occurring sequence. Thus, the term "recombinant" polypeptide does not necessarily refer to a polypeptide whose sequence does not naturally occur. Instead, a "recombinant" polypeptide is encoded by a recombinant DNA sequence, but the sequence of the polypeptide can be naturally occurring ("wild type") or non-naturally occurring (e.g., a variant, a mutant, etc.). Thus, a "recombinant" polypeptide is the result of human intervention, but may be a naturally occurring amino acid sequence.

[0037] A "vector" or "expression vector" is a replicon, such as plasmid, phage, virus, or cosmid, to which another DNA segment, i.e. an "insert", may be attached so as to bring about the replication of the attached segment in a cell.

[0038] An "expression cassette" comprises a DNA coding sequence operably linked to a promoter. "Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

[0039] The terms "recombinant expression vector," or "DNA construct" are used interchangeably herein to refer to a DNA molecule comprising a vector and one insert. Recombinant expression vectors are usually generated for the purpose of expressing and/or propagating the insert(s), or for the construction of other recombinant nucleotide sequences. The insert(s) may or may not be operably linked to a promoter sequence and may or may not be operably linked to DNA regulatory sequences.

[0040] Any given component, or combination of components can be unlabeled, or can be detectably labeled with a label moiety. In some cases, when two or more components are labeled, they can be labeled with label moieties that are distinguishable from one another.

[0041] General methods in molecular and cellular biochemistry can be found in such standard textbooks as *Molecular Cloning: A Laboratory Manual*, 3rd Ed. (Sambrook et al., HaRBor Laboratory Press 2001); *Short Protocols in Molecular Biology*, 4th Ed. (Ausubel et al. eds., John

Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds., Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998), the disclosures of which are incorporated herein by reference.

- [0042]** Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.
- [0043]** Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.
- [0044]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.
- [0045]** It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an RNA-guided effector polypeptide” includes a plurality of such RNA-guided effector polypeptides and reference to “the guide RNA” includes reference to one or more guide RNAs and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

- [0046] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.
- [0047] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

- [0048] The present disclosure provide an RNA-guided effector polypeptide having a length that is less than *Streptococcus pyogenes* Cas9, and that retains the ability, when complexed with a guide RNA, to bind to a target nucleic acid. The present disclosure provides a fusion polypeptide comprising: i) an RNA-guided effector polypeptide of the present disclosure; and ii) a fusion partner. The present disclosure further provides nucleic acids encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. Methods of using an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure are also provided.
- [0049] An RNA-guided effector polypeptide of the present disclosure has a length that is less than *Streptococcus pyogenes* Cas9 (Spy Cas9). An RNA-guided effector polypeptide that has a length that is less than Spy Cas9 is advantageous. Expression vectors comprising a nucleotide sequence encoding a heterologous polypeptide such as Cas9 have size limitations; such expression vectors can more readily accommodate an RNA-guided effector polypeptide that has a length less than the 1,368-amino acid full-length Spy Cas9. Moreover, a smaller RNA-guided effector polypeptide can serve as a scaffold for a fusion polypeptide comprising the RNA-guided effector polypeptide and a fusion partner, where the fusion partner provides a functionality such as nuclease activity, transcriptional control, base editing, and the like. An expression vector can also accommodate a nucleotide sequence encoding such a fusion polypeptide, due to the smaller size of the RNA-guided effector polypeptide.

RNA-GUIDED EFFECTOR POLYPEPTIDES

- [0050]** The present disclosure provides an RNA-guided effector polypeptide, where the RNA-guided effector polypeptide has a length that is less than 1,368 amino acids, and then retains the ability, when complexed with a guide RNA, to bind to a target nucleic acid.
- [0051]** An RNA-guided effector polypeptide of the present disclosure can have a length of from about 700 amino acids to about 1000 amino acids, e.g., from about 700 amino acids (aa) to about 705 aa, from about 705 aa to about 710 aa, from about 710 aa to about 715 aa, from about 715 aa to about 720 aa, from about 720 aa to about 725 aa, from about 725 aa to about 730 aa, from about 730 aa to about 735 aa, from about 735 aa to about 740 aa, from about 740 aa to about 745 aa, from about 745 aa to about 750 aa, from about 750 aa to about 755 aa, from about 755 aa to about 760 aa, from about 760 aa to about 765 aa, from about 765 aa to about 770 aa, from about 770 aa to about 775 aa, from about 775 aa to about 780 aa, from about 780 aa to about 785 aa, from about 785 aa to about 790 aa, from about 790 aa to about 800 aa, from about 800 aa to about 810 aa, from about 810 aa to about 820 aa, from about 820 aa to about 830 aa, from about 830 aa to about 840 aa, from about 840 aa to about 850 aa, from about 850 aa to about 860 aa, from about 860 aa to about 870 aa, from about 870 aa to about 880 aa, from about 880 aa to about 890 aa, from about 890 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa.
- [0052]** In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 750 amino acids to about 950 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 800 amino acids to about 950 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 850 amino acids to about 950 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 850 amino acids to about 1000 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 850 amino acids to about 925 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 850 amino acids to about 900 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 900 amino acids to about 1000 amino acids. In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 950 amino acids to about 1000 amino acids.
- [0053]** In some cases, an RNA-guided effector polypeptide of the present disclosure has a length of from about 1000 amino acids to about 1200 amino acids, e.g., from about 1000 amino acids (aa) to about 1050 aa, from about 1050 aa to about 1100 aa, from about 1100 aa to about 1150 aa, or from about 1150 aa to about 1200 aa. In some cases, an RNA-guided effector polypeptide of the

present disclosure has a length of from about 1200 amino acids to about 1364 amino acids, e.g., from about 1200 amino acids (aa) to about 1210 aa, from about 1210 aa to about 1220 aa, from about 1220 aa to about 1230 aa, from about 1230 aa to about 1240 aa, from about 1240 aa to about 1250 aa, from about 1250 aa to about 1260 aa, from about 1260 aa to about 1270 aa, from about 1270 aa to about 1280 aa, from about 1280 aa to about 1290 aa, from about 1290 aa to about 1300 aa, from about 1300 aa to about 1310 aa, from about 1310 aa to about 1320 aa, from about 1320 aa to about 1330 aa, from about 1330 aa to about 1340 aa, from about 1340 aa to about 1350 aa, or from about 1350 aa to about 1365 aa.

- [0054]** An RNA-guided effector polypeptide of the present disclosure will in some cases exhibit reduced nuclease activity, compared to the nuclease activity exhibited by a Spy Cas9 polypeptide comprising the amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure exhibits 50% or less, 40% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 5% or less, 2% or less, or 1% or less, of the nuclease activity exhibited by a Spy Cas9 polypeptide comprising the amino acid sequence depicted in FIG. 1. Methods for determining nuclease activity of an RNA-guided effector polypeptide are well known in the art, and any such method can be used to determine whether an RNA-guided effector polypeptide of the present disclosure exhibits reduced nuclease activity, compared to the nuclease activity exhibited by a Spy Cas9 polypeptide comprising the amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure does not exhibit detectable nuclease activity.
- [0055]** An RNA-guided effector polypeptide of the present disclosure, when complexed with a guide RNA, retains the ability to bind to a target nucleic acid, where the guide RNA comprises a nucleotide sequence that is complementary to a nucleotide sequence in the target nucleic acid. In some cases, an RNA-guided effector polypeptide of the present disclosure, when complexed with a guide RNA, exhibits at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more than 95%, of the binding affinity for a target nucleic acid of a Spy Cas9 polypeptide comprising the amino acid sequence depicted in FIG. 1, when complexed with the same guide RNA, to the same target nucleic acid.
- [0056]** In some cases, an RNA-guided effector polypeptide of the present disclosure comprises 1, 2, 3, or 4 deletions, relative to a Cas9 polypeptide having at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Spy Cas9 amino acid sequence depicted in FIG. 1. Each of the 1, 2, 3, or 4 deletions can be from 4 contiguous amino acids to 255 contiguous amino acids in length.
- [0057]** In some cases, RNA-guided effector polypeptide of the present disclosure comprises one or more of:

- [0058] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324 (referred to herein as an “A” deletion);
- [0059] ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739 (referred to herein as a “B” deletion);
- [0060] iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981 (referred to herein as a “C” deletion); and
- [0061] d) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112 (referred to herein as a “D” deletion),
- [0062] where the amino acid numbering is based on the amino acid numbering of the Spy Cas9 amino acid sequence depicted in FIG. 1. An RNA-guided effector polypeptide of the present disclosure can comprise one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids of the Spy Cas9 amino acid sequence depicted in FIG. 1.
- [0063] Suitable start and end points for the deletions are depicted in FIG. 2A and 2B. Any “start” and “end” sites within “A” deletion can be combined to generate an “A” deletion. Any “start” and “end” sites within “B” deletion can be combined to generate a “B” deletion. Any “start” and “end” sites within “C” deletion can be combined to generate a “C” deletion. Any “start” and “end” sites within “D” deletion can be combined to generate a “D” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only an “A” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “B” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “C” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “D” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only an “A” deletion and a “B” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only an “A” deletion and a “C” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only an “A” deletion and a “D” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “B”

deletion and a “C” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “B” deletion and a “D” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only a “C” deletion and a “D” deletion. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion, a “B” deletion, and a “C” deletion (and not a “D” deletion). In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion, a “B” deletion, and a “D” deletion (and not a “C” deletion). In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion, a “D” deletion, and a “D” deletion (and not a “B” deletion). In some cases, an RNA-guided effector polypeptide of the present disclosure comprises “B” deletion, a “C” deletion, and a “D” deletion (and not an “A” deletion). In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion, a “B” deletion, a “C” deletion, and a “D” deletion.

[0064] As depicted in FIG. 8, in some cases, an “A” deletion comprises all or a portion of the Helical II domain of Spy Cas9. As depicted in FIG. 8, in some cases, a “B” deletion comprises all or a portion of the Helical III domain of Spy Cas9. As depicted in FIG. 8, in some cases, a “C” deletion comprises all or a portion of the HNH domain of Spy Cas9; and may also comprises a portion of the RuvC III domain of Spy Cas9. As depicted in FIG. 8, in some cases, a “D” deletion comprises a portion of the RuvC III domain of Spy Cas9.

[0065] Non-limiting examples of RNA-guided effector polypeptides of the present disclosure that comprises one or more of “A,” “B,” “C,” and “D” deletions are depicted in FIG. 23. Examples of suitable combination of “A,” “B,” “C,” and “D” deletions are depicted in FIG. 23.

[0066] In some cases, an RNA-guided effector polypeptide of the present disclosure is a Cas9 protein that lacks all or a portion of one or more of following domains (in any combination): the Helical II domain, the Helical III domain, the HNH domain, and the RuvC III domain. Any Cas9 protein can be used as a starting point for generation deletions, to generate an RNA-guided effector polypeptide of the present disclosure. Examples of suitable Cas9 proteins include, but are not limited to, those set forth in SEQ ID NOs: 5-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion and a “B” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion and a “C” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-

816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion and a “D” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion, a “B” deletion, and a “C” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion a “B” deletion, a “C” deletion, and a “D” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises a “B” deletion, a “C” deletion, and a “D” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises an “A” deletion a “C” deletion and a “D” deletion based on the amino acid sequence of SEQ ID NO:5, or a corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs: 6-816. While the following description of various deletions refers to Spy Cas9, the description can be applied to a Cas9 comprising an amino acid sequence set forth in any one of SEQ ID NOs:5-816. The amino acid sequence of a “corresponding deletion of a polypeptide as set forth in any one of SEQ ID NOs:6-816” can be readily determined using a multiple sequence alignment of one or more of the amino acid sequences set forth in SEQ ID NOs:6-816 with the amino acid sequence set forth in SEQ ID NO:5 (*Streptococcus pyogenes* Cas9).

“A” deletion

[0067] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700

to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only one deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion has a length of from about 1210 amino acids to about 1363 amino acids, e.g., from about 1210 amino acids (aa) to about 1220 aa, from about 1220 aa to about 1230 aa, from about 1230 aa to about 1240 aa, from about 1240 aa to about 1250 aa, from about 1250 aa to about 1260 aa, from about 1260 aa to about 1270 aa, from about 1270 aa to about 1280 aa, from about 1280 aa to about 1290 aa, from about 1290 aa to about 1300 aa, from about 1300 aa to about 1310 aa, from about 1310 aa to about 1320 aa, from about 1320 aa to about 1330 aa, from about 1330 aa to about 1340 aa, from about 1340 aa to about 1350 aa, or from about 1350 aa to 1363 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only an “A” deletion has a length of 1210 amino acids.

“B” deletion

[0068] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only one deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about

150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion has a length of from about 1113 amino acids to about 1338 amino acids, e.g., from about 1113 amino acids (aa) to about 1120 aa, from about 1120 aa to about 1130 aa, from about 1130 aa to about 1140 aa, from about 1140 aa to about 1150 aa, from about 1150 aa to about 1160 aa, from about 1160 aa to about 1170 aa, from about 1170 aa to about 1180 aa, from about 1180 aa to about 1190 aa, from about 1190 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to 1338 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only a “B” deletion has a length of 1163 aa, 1164 aa, or 1165 aa.

“C” deletion

[0069] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only one deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “C” deletion has a length of from about 1148 amino acids to

about 1364 amino acids, e.g., from about 1148 amino acids (aa) to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, from about 1325 aa to 1350 aa, or from about 1350 aa to 1364 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only a “C” deletion has a length of 1263 aa, 1264 aa, or 1265 aa.

“D” deletion

[0070] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprises only one deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “D” deletion has a length of from about 1250 amino acids to about 1348 amino acids, e.g., from about 1250 amino acids (aa) to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to about 1348 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only a “D” deletion has a length of 1297 aa, 1298 aa, or 1299 aa.

“A + B” deletion

[0071] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from

about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A” and “B” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “B” deletion has a length of from about 955 amino acids to about 1333 amino acids, e.g., from about 955 amino acids (aa) to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1333 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only an “A” deletion and a “B” deletion has a length of from about 1045 aa to about 1050 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only an “A” deletion and a “B” deletion has a length of 1046 aa, 1047 aa, or 1048 aa.

“A + C” deletion

[0072] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to

amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A” and “C” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “C” deletion has a length of from about 990 amino acids to about 1360 amino acids, e.g., from about 990 amino acids (aa) to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to about 1360 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising only an “A” deletion and a “C” deletion has a length of from about 1140 aa to about 1150 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “C” deletion has a length of 1146 aa, 1147 aa, 1148 aa, 1149 aa, or 1150 aa.

“A + D” deletion

[0073] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion

of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “D” deletion has a length of from about 1090 amino acids to about 1343 amino acids, e.g., from about 1090 amino acids (aa) to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to about 1343 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “D” deletion has a length of from about 1275 aa to about 1300 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion and a “D” deletion has a length of 1280 aa, 1281 aa, 1282 aa, 1283 aa, or 1284 aa.

“B” + “C” deletions

[0074] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150

to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “B” and “C” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “C” deletion has a length of from about 890 amino acids to about 1334 amino acids, e.g., from about 890 amino acids (aa) to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to about 1334 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “C” deletion has a length of from about 1050 aa to about 1075 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “C” deletion has a length of 1058 aa, 1059 aa, 1060 aa, 1061 aa, or 1062 aa.

“B” + “D” deletion

[0075] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from

about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “B” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “D” deletion has a length of from about 995 amino acids to about 1318 amino acids, e.g., from about 995 amino acids (aa) to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1318 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “D” deletion has a length of from about 1090 aa to about 1100 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion and a “D” deletion has a length of 1092 aa, 1093 aa, 1094 aa, or 1095 aa.

“C” + “D” deletions

- [0076]** In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and ii) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9

amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “C” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “C” deletion and a “D” deletion has a length of from about 1030 amino acids to about 1344 amino acids, e.g., from about 1030 amino acids (aa) to about 1050 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to about 1344 aa. . In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “C” deletion and a “D” deletion has a length of from about 1190 to about 1200. . In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “C” deletion and a “D” deletion has a length of 1192 aa, 1193 aa, 1194 aa, or 1195 aa.

“A” + “B” + “C” deletions

[0077] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; ii) a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and iii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids

761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A,” “B,” and “C” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “C” deletion has a length of from about 735 amino acids to about 1329 amino acids, e.g., from about 735 amino acids (aa) to about 750 aa, from about 750 aa to about 775 aa, from about 775 aa to about 800 aa, from about 800 aa to about 825 aa, from about 825 aa to about 850 aa, from about 850 aa to about 875 aa, from about 875 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1329 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “C” deletion has a length of from about 930 aa to about 970 aa. . In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “C” deletion has a length of from about 940 aa to about 950 aa.

“A” + “B” + “D” deletions

[0078] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; ii) a deletion of from about 30 contiguous amino acids to about 255 contiguous amino acids (e.g., from about 30

to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 255 contiguous amino acids) of a region corresponding to amino acids 484-739 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and iii) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A,” “B,” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “D” deletion has a length of from about 837 amino acids to about 1313 amino acids, e.g., from about 837 amino acids (aa) to about 850 aa, from about 850 aa to about 875 aa, from about 875 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1313 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “D” deletion has a length of from about 970 aa to about 1000 aa, In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, and a “D” deletion has a length of from about 975 aa to about 980 aa.

“A” + “C” + “D” deletions

[0079] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; ii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and iii) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “A,” “C,” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “C” deletion, and a “D” deletion has a length of from about 870 amino acids to about 1340 amino acids, e.g., from about 870 amino acids (aa) to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, from about 1300 aa to about 1325 aa, or from about 1325 aa to

about 1340 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “C” deletion, and a “D” deletion has a length of from about 1070 aa to about 1100 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “C” deletion, and a “D” deletion has a length of from about 1075 aa to about 1080 aa.

“B” + “C” + “D” deletions

[0080] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; ii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and iii) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1. In some case, the RNA-guided effector polypeptide comprises no deletions other than the “B,” “C,” and “D” deletions. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 775 amino acids to about 1314 amino acids, e.g., from about 775 aa to about 800 aa, from about 800 aa to about 825 aa, from about 825 aa to about 850 aa, from about 850 aa to about 875 aa, from about 875 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950

aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1314 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 980 aa to about 1000 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 980 aa to about 990 aa.

“A” + “B” + “C” + “D” deletions

[0081] In some cases, an RNA-guided effector polypeptide of the present disclosure comprises: i) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; ii) a deletion of from about 5 contiguous amino acids to about 158 contiguous amino acids (e.g., from about 5 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 125, or from about 125 to about 158 contiguous amino acids) of a region corresponding to amino acids 166-324 of the Spy Cas9 amino acid sequence depicted in FIG. 1; iii) a deletion of from about 4 contiguous amino acids to about 220 contiguous amino acids (e.g., from about 4 to about 25, from about 25 to about 50, from about 50 to about 75, from about 75 to about 100, from about 100 to about 150, from about 150 to about 200, or from about 200 to about 220, contiguous amino acids) of a region corresponding to amino acids 761-981 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and iv) a deletion of from about 20 contiguous amino acids to about 118 contiguous amino acids (e.g., from about 20 to about 40, from about 40 to about 60, from about 60 to about 80, from about 80 to about 100, or from about 100 to about 118 contiguous amino acids) of a region corresponding to amino acids 994-1112 of the Spy Cas9 amino acid sequence depicted in FIG. 1; and comprises one or more stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200, from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to corresponding stretches of from 50 to 1000 contiguous amino acids (e.g., from 50 to 75, from 75 to 100, from 100 to 150, from 150 to 200,

from 200 to 250, from 250 to 300, from 300 to 350, from 350 to 400, from 400 to 450, from 450 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, or from 900 to 1000, contiguous amino acids) of the Spy Cas9 amino acid sequence depicted in FIG. 1.

[0082] In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 615 amino acids to about 1310 amino acids, e.g., from about 615 amino acids (aa) to about 625 aa, from about 625 aa to about 650 aa, from about 650 aa to about 675 aa, from about 675 aa to about 700 aa, from about 700 aa to about 725 aa, from about 725 aa to about 750 aa, from about 750 aa to about 775 aa, from about 775 aa to about 800 aa, from about 800 aa to about 825 aa, from about 825 aa to about 850 aa, from about 850 aa to about 875 aa, from about 875 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, from about 975 aa to about 1000 aa, from about 1000 aa to about 1025 aa, from about 1025 aa to about 1050 aa, from about 1050 aa to about 1075 aa, from about 1075 aa to about 1100 aa, from about 1100 aa to about 1125 aa, from about 1125 aa to about 1150 aa, from about 1150 aa to about 1175 aa, from about 1175 aa to about 1200 aa, from about 1200 aa to about 1225 aa, from about 1225 aa to about 1250 aa, from about 1250 aa to about 1275 aa, from about 1275 aa to about 1300 aa, or from about 1300 aa to about 1310 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 870 aa to about 1000 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 870 aa to about 900 aa. In some cases, an RNA-guided effector polypeptide of the present disclosure comprising an “A” deletion, a “B” deletion, a “C” deletion, and a “D” deletion has a length of from about 870 aa to about 875 aa.

Examples of RNA-guided effector polypeptides

[0083] As an example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the D3CE polypeptide depicted in FIG. 3. The D3CE polypeptide depicted in FIG. 3 comprises a “B” deletion of amino acids 503-708, a “C” deletion of amino acids 792-897, and a “D” deletion of amino acids 1010-1081, compared to the amino acid numbering of the SpyCas9 amino acid sequence set forth in FIG. 1. The locations of the deletions, relative to the SpyCas9 amino acid sequence set forth in FIG. 1, are depicted in FIG. 5A-5B. D3CE has a length of 987 amino acids.

[0084] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least

95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the D4CE polypeptide depicted in FIG. 4. The D4CE polypeptide depicted in FIG. 4 comprises an “A” deletion of amino acids 179-296, a “B” deletion of amino acids 503-708, a “C” deletion of amino acids 792-897, and a “D” deletion of amino acids 1010-1081, compared to the amino acid numbering of the SpyCas9 amino acid sequence set forth in FIG. 1. The locations of the deletions, relative to the SpyCas9 amino acid sequence set forth in FIG. 1, are depicted in FIG. 6A-6B. D4CE has a length of 874 amino acids.

- [0085]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 1 polypeptide depicted in FIG. 10B. As depicted in FIG. 10A, a Variant 1 polypeptide has an “A” deletion of amino acids 174-289 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1253 amino acids.
- [0086]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 2 polypeptide depicted in FIG. 11B. As depicted in FIG. 11A, a Variant 2 polypeptide has an “A” deletion of amino acids 174-306 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1235 amino acids.
- [0087]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 3 polypeptide depicted in FIG. 12B. As depicted in FIG. 12A, a Variant 3 polypeptide has an “A” deletion of amino acids 175-296 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1246 amino acids.
- [0088]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 4 polypeptide depicted in FIG. 13B. As depicted in FIG. 13A, a Variant 4 polypeptide has a “B” deletion of amino acids 522-702 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1187 amino acids.
- [0089]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid

sequence of the Variant 5 polypeptide depicted in FIG. 14B. As depicted in FIG. 14A, a Variant 5 polypeptide has a “B” deletion of amino acids 537-704 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1200 amino acids.

[0090] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 6 polypeptide depicted in FIG. 15B. As depicted in FIG. 15A, a Variant 6 polypeptide has a “B” deletion of amino acids 523-664 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1226 amino acids.

[0091] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 7 polypeptide depicted in FIG. 16B. As depicted in FIG. 16A, a Variant 7 polypeptide has a “C” deletion of amino acids 781-906 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1242 amino acids.

[0092] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 8 polypeptide depicted in FIG. 17B. As depicted in FIG. 17A, a Variant 8 polypeptide has a “C” deletion of amino acids 820-932 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1255 amino acids.

[0093] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 9 polypeptide depicted in FIG. 18B. As depicted in FIG. 18A, a Variant 9 polypeptide has a “C” deletion of amino acids 769-910 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1226 amino acids.

[0094] As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 10 polypeptide depicted in FIG. 19B. As depicted in FIG. 19A, a Variant 10 polypeptide has: i) a “B” deletion of amino acids 508-649; ii) a “C” deletion of amino acids 768-900; and iii) a “D” deletion of amino acids 1010-1081 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1021 amino acids.

- [0095]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 11 polypeptide depicted in FIG. 20B. As depicted in FIG. 20A, a Variant 11 polypeptide has: i) a “B” deletion of amino acids 508-646; ii) a “C” deletion of amino acids 786-923; and iii) a “D” deletion of amino acids 1010-1081 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1019 amino acids.
- [0096]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 12 polypeptide depicted in FIG. 21B. As depicted in FIG. 21A, a Variant 12 polypeptide has: i) a “B” deletion of amino acids 509-650; ii) a “C” deletion of amino acids 776-923; and iii) a “D” deletion of amino acids 1010-1081 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 1006 amino acids.
- [0097]** As another example, in some cases, an RNA-guided effector polypeptide of the present disclosure comprises an amino acid sequence having at least at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence of the Variant 13 polypeptide depicted in FIG. 22B. As depicted in FIG. 22A, a Variant 13 polypeptide has: i) a “B” deletion of amino acids 504-710; ii) a “C” deletion of amino acids 763-924; and iii) a “D” deletion of amino acids 1010-1081 compared to the Spy Cas9 polypeptide depicted in FIG. 1; and has a length of about 927 amino acids.

FUSION POLYPEPTIDES COMPRISING AN RNA-GUIDED EFFECTOR POLYPEPTIDE

- [0098]** The present disclosure provides a fusion polypeptide comprising: i) an RNA-guided effector polypeptide of the present disclosure; and ii) a heterologous fusion partner.
- [0099]** In some cases, the heterologous fusion partner is a polypeptide having a length of no more than 500 amino acids (aa), no more than 400 aa, no more than 300 aa, no more than 200 aa, or no more than 100 aa. In some cases, the heterologous fusion partner is a polypeptide having a length of from about 10 amino acids (aa) to about 25 aa, from about 25 aa to about 50 aa, from about 50 aa to about 100 aa, from about 100 aa to about 150 aa, from about 150 aa to about 200 aa, from about 200 aa to about 300 aa, from about 300 aa to about 400 aa, or from about 400 aa to about 500 aa.
- [00100]** The total length of the fusion polypeptide is in some cases from about 750 amino acids to about 1500 amino acids, e.g., from about 750 amino acids (aa) to about 775 aa, from about 775 aa to about 800 aa, from about 800 aa to about 825 aa, from about 825 aa to about 850 aa,

from about 850 aa to about 875 aa, from about 875 aa to about 900 aa, from about 900 aa to about 925 aa, from about 925 aa to about 950 aa, from about 950 aa to about 975 aa, or from about 975 aa to about 1000 aa.

[00101] In other instances, the total length of the fusion polypeptide is from about 1000 amino acids to about 1500 amino acids, e.g., from about 1000 amino acids (aa) to about 1100 aa, from about 1100 aa to about 1200 aa, from about 1200 aa to about 1300 aa, from about 1300 aa to about 1400 aa, or from about 1400 aa to about 1500 aa. The total length of the fusion polypeptide is in some cases from about 1000 aa to about 1400 aa. The total length of the fusion polypeptide is in some cases from about 1000 aa to about 1300 aa. The total length of the fusion polypeptide is in some cases from about 1000 aa to about 1200 aa. The total length of the fusion polypeptide is in some cases from about 1000 aa to about 1100 aa.

[00102] Suitable heterologous fusion partners include, but are not limited to, enzymes that modify a nucleic acid; enzymes that modify a polypeptide (e.g., a polypeptide binds to a target nucleic acid); non-enzymatic polypeptides that bind to a nucleic acid; and polypeptides that modify (e.g., control) transcription.

[00103] In some cases, the heterologous fusion partner exhibits nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity. In some cases, the heterologous fusion partner exhibits nuclease activity. In some cases, the heterologous fusion partner introduces a double strand break in a target DNA. In some cases, the heterologous fusion partner modifies a target polypeptide associated with a target DNA. In some cases, the heterologous fusion partner exhibits methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, or demyristoylation activity.

[00104] In some cases, the heterologous fusion partner is a nucleic acid-modifying enzyme. Examples of suitable nucleic acid-modifying enzymes include DNA editing enzymes; deaminases, such as activation induced deaminase and APOBEC proteins; nucleases; recombinases; glycosylases; methyltransferases; and the like. In other cases the heterologous fusion partner is a protein-modifying enzyme. For example, the heterologous fusion partner may be one that modifies a protein associated with a nucleic acid. Examples of suitable protein-

modifying enzymes include histone-modifying enzymes, acetylases, kinases, methyltransferases, ubiquitin ligases, SUMO ligases, demethylases, deacetylases, phosphatases, and the like.

[00105] In some cases the fusion partner can modulate transcription (e.g., inhibit transcription, increase transcription) of a target DNA. For example, in some cases the fusion partner is a protein (or a domain from a protein) that inhibits transcription (e.g., a transcriptional repressor, a protein that functions via recruitment of transcription inhibitor proteins, modification of target DNA such as methylation, recruitment of a DNA modifier, modulation of histones associated with target DNA, recruitment of a histone modifier such as those that modify acetylation and/or methylation of histones, and the like). In some cases the fusion partner is a protein (or a domain from a protein) that increases transcription (e.g., a transcription activator, a protein that acts via recruitment of transcription activator proteins, modification of target DNA such as demethylation, recruitment of a DNA modifier, modulation of histones associated with target DNA, recruitment of a histone modifier such as those that modify acetylation and/or methylation of histones, and the like).

[00106] Examples of proteins (or fragments thereof) that can be used in increase transcription include but are not limited to: transcriptional activators such as VP16, VP64, VP48, VP160, p65 subdomain (e.g., from NFkB), and activation domain of EDLL and/or TAL activation domain (e.g., for activity in plants); histone lysine methyltransferases such as SET1A, SET1B, MLL1 to 5, ASH1, SYMD2, NSD1, and the like; histone lysine demethylases such as JHDM2a/b, UTX, JMJD3, and the like; histone acetyltransferases such as GCN5, PCAF, CBP, p300, TAF1, TIP60/PLIP, MOZ/MYST3, MORF/MYST4, SRC1, ACTR, P160, CLOCK, and the like; and DNA demethylases such as Ten-Eleven Translocation (TET) dioxygenase 1 (TET1CD), TET1, DME, DML1, DML2, ROS1, and the like.

[00107] Examples of proteins (or fragments thereof) that can be used in decrease transcription include but are not limited to: transcriptional repressors such as the Krüppel associated box (KRAB or SKD); KOX1 repression domain; the Mad mSIN3 interaction domain (SID); the ERF repressor domain (ERD), the SRDX repression domain (e.g., for repression in plants), and the like; histone lysine methyltransferases such as Pr-SET7/8, SUV4-20H1, RIZ1, and the like; histone lysine demethylases such as JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, JARID1A/RBP2, JARID1B/PLU-1, JARID1C/SMCX, JARID1D/SMCY, and the like; histone lysine deacetylases such as HDAC1, HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, SIRT1, SIRT2, HDAC11, and the like; DNA methylases such as HhaI DNA m5c-methyltransferase (M.HhaI), DNA methyltransferase 1 (DNMT1), DNA methyltransferase 3a (DNMT3a), DNA methyltransferase 3b (DNMT3b), METI, DRM3 (plants), ZMET2, CMT1,

CMT2 (plants), and the like; and periphery recruitment elements such as Lamin A, Lamin B, and the like.

[00108] In some cases, the heterologous fusion partner is a nuclease that provides for cleavage of a double-stranded DNA. In some cases, the heterologous fusion partner is a nickase. In some cases, a fusion polypeptide of the present disclosure includes a heterologous polypeptide that has enzymatic activity that modifies a target nucleic acid (e.g., nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity).

[00109] In some cases the fusion partner has enzymatic activity that modifies a protein associated with the target nucleic acid (e.g., ssRNA, dsRNA, ssDNA, dsDNA) (e.g., a histone, an RNA binding protein, a DNA binding protein, and the like). Examples of enzymatic activity (that modifies a protein associated with a target nucleic acid) that can be provided by the fusion partner include but are not limited to: methyltransferase activity such as that provided by a histone methyltransferase (HMT) (e.g., suppressor of variegation 3-9 homolog 1 (SUV39H1, also known as KMT1A), euchromatic histone lysine methyltransferase 2 (G9A, also known as KMT1C and EHMT2), SUV39H2, ESET/SETDB1, and the like, SET1A, SET1B, MLL1 to 5, ASH1, SYMD2, NSD1, DOT1L, Pr-SET7/8, SUV4-20H1, EZH2, RIZ1), demethylase activity such as that provided by a histone demethylase (e.g., Lysine Demethylase 1A (KDM1A also known as LSD1), JHDM2a/b, JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, JARID1A/RBP2, JARID1B/PLU-1, JARID1C/SMCX, JARID1D/SMCY, UTX, JMJD3, and the like), acetyltransferase activity such as that provided by a histone acetylase transferase (e.g., catalytic core/fragment of the human acetyltransferase p300, GCN5, PCAF, CBP, TAF1, TIP60/PLIP, MOZ/MYST3, MORF/MYST4, HBO1/MYST2, HMOF/MYST1, SRC1, ACTR, P160, CLOCK, and the like), deacetylase activity such as that provided by a histone deacetylase (e.g., HDAC1, HDAC2, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, HDAC9, SIRT1, SIRT2, HDAC11, and the like), kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, and demyristoylation activity.

[00110] In some cases the fusion partner has enzymatic activity that modifies the target nucleic acid (e.g., ssRNA, dsRNA, ssDNA, dsDNA). Examples of enzymatic activity that can be provided by the fusion partner include but are not limited to: nuclease activity such as that provided by a restriction enzyme (e.g., FokI nuclease), methyltransferase activity such as that

provided by a methyltransferase (e.g., HhaI DNA m5c-methyltransferase (M.HhaI), DNA methyltransferase 1 (DNMT1), DNA methyltransferase 3a (DNMT3a), DNA methyltransferase 3b (DNMT3b), MET1, DRM3 (plants), ZMET2, CMT1, CMT2 (plants), and the like); demethylase activity such as that provided by a demethylase (e.g., Ten-Eleven Translocation (TET) dioxygenase 1 (TET1CD), TET1, DME, DML1, DML2, ROS1, and the like), DNA repair activity, DNA damage activity, deamination activity such as that provided by a deaminase (e.g., a cytosine deaminase enzyme such as rat APOBEC1), dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity such as that provided by an integrase and/or resolvase (e.g., Gin invertase such as the hyperactive mutant of the Gin invertase, GinH106Y; human immunodeficiency virus type 1 integrase (IN); Tn3 resolvase; and the like), transposase activity, recombinase activity such as that provided by a recombinase (e.g., catalytic domain of Gin recombinase), polymerase activity, ligase activity, helicase activity, photolyase activity, and glycosylase activity).

[00111] As one non-limiting example, in some cases, the heterologous fusion partner: i) is a FokI nuclease comprising an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the FokI polypeptide depicted in FIG. 7A or FIG. 7B; and ii) has a length of from about 195 amino acids to about 200 amino acids.

[00112] In some cases, the heterologous fusion partner is a deaminase. Suitable deaminases include a cytidine deaminase and an adenosine deaminase.

[00113] In some cases, a suitable deaminase provides for generation of a G → T substitution. In some cases, a suitable deaminase provides for generation of a G → C substitution. In some cases, a suitable deaminase provides for generation of a C → A substitution. In some cases, a suitable deaminase provides for generation of a C → G substitution. In some cases, a suitable deaminase provides for generation of an A → T substitution. In some cases, a suitable deaminase provides for generation of an A → G substitution. In some cases, a suitable deaminase provides for generation of an A → C substitution. In some cases, a suitable deaminase provides for generation of a T → A substitution. In some cases, a suitable deaminase provides for generation of a T → G substitution. In some cases, a suitable deaminase provides for generation of a T → C substitution. In some cases, a suitable deaminase provides for generation of a G → A substitution. In some cases, a suitable deaminase provides for generation of a C → T substitution.

[00114] Adenosine deaminases suitable as heterologous fusion partners include any enzyme that is capable of deaminating adenosine in DNA. In some cases, the deaminase is a TadA deaminase.

[00115] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAH
AEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAA
GSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTD (SEQ ID
NO:1)

[00116] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWN
RPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFG
ARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQ
SSTD (SEQ ID NO: 2).

[00117] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Staphylococcus aureus* TadA amino acid sequence:

MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETLQQPTAHAE
HIAIERAAKVLGSRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKGGCSGSL
MNLLQQSNFNHRAIVDKGVLKEACSTLLTFFK NLRANKKSTN: (SEQ ID NO:3)

[00118] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Bacillus subtilis* TadA amino acid sequence:

MTQDELYMKEAIKEAKKAEKGEVPIGAVLVINGEIIARAHNLRETEQRSIAHAEMLVID
EACKALGTWRLEGATLYVTLEPCPMCAVAVLSRVEKVVFGAFDPKGGCSGTLMNLL
QEERFNHQAEEVSGVLEEECGGMLSAFFRELKKKKAARKNLSE (SEQ ID NO: 4)

[00119] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at

least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Salmonella typhimurium* TadA:

MPPAFITGVTSLSDELVDHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWN
RPIGRHDPTAHAEIMALRQGGLVLQNYRLDITLYVTLEPCVMCAGAMVHSRIGRVVFG
GARDAKTGAAGSLIDVLHHPGMNHRVEIEGVLRDECATLLSDFFRMRRQEIKALKKAD
RAEGAGPAV (SEQ ID NO: 817).

[00120] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Shewanella putrefaciens* TadA amino acid sequence:
 MDEYWMQVAMQMAEKAEAAAGEVPVGAVLVKDGQQIATGYNLSISQHDPTAHAEILCL
 RSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEKTGAAGTVVNL
 LQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQQGIE (SEQ ID NO: 818)

[00121] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Haemophilus influenzae* F3031 TadA amino acid sequence:
 MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGWNLSIVQSDP
 TAHAEIILRNAGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVFGASDYKTGAI
 GSRFHFFDDYKMNHTLEITSGVLAEESQKLS TFFQKRREEKKIEKALLKSLSDK (SEQ ID NO: 819)

[00122] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Caulobacter crescentus* TadA amino acid sequence:
 MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAGNGPIAAHDP
 TAHAEIAAMRAAAAKLGNYRLTDLTLVVTLTPCAMCAGAISHARIGRVVFGADDPKGG
 AVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI (SEQ ID NO: 820)

[00123] In some cases, a suitable adenosine deaminase comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following *Geobacter sulfurreducens* TadA amino acid sequence:
 MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHNLREGSNDP
 SAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAILARLERVVFGCYDPKGG
 AAGSLYDLSADPRLNHQVRLSPGVCQEECTMLSDFFRDLLRRRKAKATPALFIDERKV
 PPEP (SEQ ID NO: 821)

[00124] Cytidine deaminases suitable as heterologous fusion partners include any enzyme that is capable of deaminating cytidine in DNA.

[00125] In some cases, the cytidine deaminase is a deaminase from the apolipoprotein B mRNA-editing complex (APOBEC) family of deaminases. In some cases, the APOBEC family deaminase is selected from the group consisting of APOBEC1 deaminase, APOBEC2 deaminase, APOBEC3A deaminase, APOBEC3B deaminase, APOBEC3C deaminase, APOBEC3D

deaminase, APOBEC3F deaminase, APOBEC3G deaminase, and APOBEC3H deaminase. In some cases, the cytidine deaminase is an activation induced deaminase (AID).

[00126] In some cases, a suitable cytidine deaminase comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence:

[00127] MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNKGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHENSVRLSRQLRRILLPLYEVDDLRLDAFRTLGL (SEQ ID NO:822)

[00128] In some cases, a suitable cytidine deaminase is an AID and comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: MDSLLMNRRKFLYQFKNVRW AKGRRETYLC YVVKRRDSAT SFSLDFGYLR NKNKGCHVELLFLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVADFLRG NPNLSLRIFTARLYFCEDRK AEPEGLRRLH RAGVQIAIMT FKENHERTFK AWEGLHENSVRLSRQLRRIL LPLYEVDDLRL DAFRTLGL (SEQ ID NO:823).

[00129] In some cases, a suitable cytidine deaminase is an AID and comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the following amino acid sequence: MDSLLMNRRKFLYQFKNVRW AKGRRETYLC YVVKRRDSAT SFSLDFGYLR NKNKGCHVELLFLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVADFLRG NPNLSLRIFTARLYFCEDRK AEPEGLRRLH RAGVQIAIMT FKDYFYCWNT FVENHERTFK AWEGLHENSVRLSRQLRRIL LPLYEVDDLRL DAFRTLGL (SEQ ID NO:824).

Additional polypeptides

[00130] In some cases, a fusion polypeptide of the present disclosure comprises one or more additional polypeptides, where suitable additional polypeptides include, e.g., a nuclear localization signal (NLS), a cell-penetrating peptide (CPP), an endosomolytic peptide, and the like. In some cases, a heterologous polypeptide (a fusion partner) provides for subcellular localization, i.e., the heterologous polypeptide contains a subcellular localization sequence (e.g., a nuclear localization signal (NLS) for targeting to the nucleus, a sequence to keep the fusion protein out of the nucleus, e.g., a nuclear export sequence (NES), a sequence to keep the fusion protein retained in the cytoplasm, a mitochondrial localization signal for targeting to the mitochondria, a chloroplast localization signal for targeting to a chloroplast, an ER retention signal, and the like). In some cases, a fusion polypeptide of the present disclosure does not

include a NLS so that the protein is not targeted to the nucleus. In some cases, the heterologous polypeptide can provide a tag (i.e., the heterologous polypeptide is a detectable label) for ease of tracking and/or purification (e.g., a fluorescent protein, e.g., green fluorescent protein (GFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), cyan fluorescent protein (CFP), mCherry, tdTomato, and the like; a histidine tag, e.g., a 6XHis tag; a hemagglutinin (HA) tag; a FLAG tag; a Myc tag; and the like).

[00131] In some cases, a fusion polypeptide of the present disclosure includes (is fused to) a nuclear localization signal (NLS) (e.g., in some cases 2 or more, 3 or more, 4 or more, or 5 or more NLSs). Thus, in some cases, a fusion polypeptide of the present disclosure includes one or more NLSs (e.g., 2 or more, 3 or more, 4 or more, or 5 or more NLSs). In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the N-terminus and/or the C-terminus. In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the N-terminus. In some cases, one or more NLSs (2 or more, 3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) the C-terminus. In some cases, one or more NLSs (3 or more, 4 or more, or 5 or more NLSs) are positioned at or near (e.g., within 50 amino acids of) both the N-terminus and the C-terminus. In some cases, an NLS is positioned at the N-terminus and an NLS is positioned at the C-terminus.

[00132] Non-limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV (SEQ ID NO: 825); the NLS from nucleoplasmin (e.g., the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK (SEQ ID NO: 826)); the c-myc NLS having the amino acid sequence PAAKRVKLD (SEQ ID NO: 827) or RQRRNELKRSP (SEQ ID NO: 828); the hRNPA1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 829); the sequence RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 830) of the IBB domain from importin-alpha; the sequences VSRKRPRP (SEQ ID NO: 831) and PPKKARED (SEQ ID NO: 832) of the myoma T protein; the sequence PQPKKKPL (SEQ ID NO: 833) of human p53; the sequence SALIKKKKKMAP (SEQ ID NO: 834) of mouse c-abl IV; the sequences DRLRR (SEQ ID NO: 835) and PKQKKRK (SEQ ID NO: 836) of the influenza virus NS1; the sequence RKLKKKIKKL (SEQ ID NO: 837) of the Hepatitis virus delta antigen; the sequence REKKKFLKRR (SEQ ID NO: 838) of the mouse Mx1 protein; the sequence KRKGDEV DGVDEVAKKKSKK (SEQ ID NO: 839) of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLEARKTKK (SEQ ID NO: 840) of the steroid hormone receptors (human) glucocorticoid. In general, NLS (or multiple NLSs) are of sufficient

strength to drive accumulation of the protein in a detectable amount in the nucleus of a eukaryotic cell. Detection of accumulation in the nucleus may be performed by any suitable technique.

[00133] In some cases, a fusion polypeptide of the present disclosure comprises: a) an RNA-guided effector polypeptide of the present disclosure; and b) a chloroplast transit peptide. Thus, for example, an RNA-guided effector polypeptide/guide RNA complex can be targeted to the chloroplast. In some cases, this targeting may be achieved by the presence of an N-terminal extension, called a chloroplast transit peptide (CTP) or plastid transit peptide. Chromosomal transgenes from bacterial sources must have a sequence encoding a CTP sequence fused to a sequence encoding an expressed polypeptide if the expressed polypeptide is to be compartmentalized in the plant plastid (e.g. chloroplast). Accordingly, localization of an exogenous polypeptide to a chloroplast is often accomplished by means of operably linking a polynucleotide sequence encoding a CTP sequence to the 5' region of a polynucleotide encoding the exogenous polypeptide. The CTP is removed in a processing step during translocation into the plastid. Processing efficiency may, however, be affected by the amino acid sequence of the CTP and nearby sequences at the N-terminus of the peptide. Other options for targeting to the chloroplast which have been described are the maize cab-m7 signal sequence (U.S. Pat. No. 7,022,896, WO 97/41228) a pea glutathione reductase signal sequence (WO 97/41228) and the CTP described in US2009029861.

[00134] In some cases, a fusion polypeptide of the present disclosure can comprise: a) an RNA-guided effector polypeptide of the present disclosure; and b) an endosomal escape peptide. In some cases, an endosomal escape polypeptide comprises the amino acid sequence GLFXALLXLLXSLWXLLLXA (SEQ ID NO:841), wherein each X is independently selected from lysine, histidine, and arginine. In some cases, an endosomal escape polypeptide comprises the amino acid sequence GLFHALLHLLHSLWHLLLHA (SEQ ID NO:842).

[00135] In some instances, an RNA-guided effector polypeptide of the present disclosure is fused to a fusion partner via a linker polypeptide (e.g., one or more linker polypeptides). The linker polypeptide may have any of a variety of amino acid sequences. Proteins can be joined by a spacer peptide, generally of a flexible nature, although other chemical linkages are not excluded. Suitable linkers include polypeptides of between 4 amino acids and 40 amino acids in length, or between 4 amino acids and 25 amino acids in length. These linkers can be produced by using synthetic, linker-encoding oligonucleotides to couple the proteins, or can be encoded by a nucleic acid sequence encoding the fusion protein. Peptide linkers with a degree of flexibility can be used. The linking peptides may have virtually any amino acid sequence, bearing in mind that the preferred linkers will have a sequence that results in a generally flexible peptide. The use

of small amino acids, such as glycine and alanine, are of use in creating a flexible peptide. The creation of such sequences is routine to those of skill in the art. A variety of different linkers are commercially available and are considered suitable for use.

[00136] Examples of linker polypeptides include glycine polymers, glycine-serine polymers (including, for example, GSGGS (SEQ ID NO: 843), GGSGGS (SEQ ID NO: 844), and GGGS (SEQ ID NO: 845), glycine-alanine polymers, alanine-serine polymers. Exemplary linkers can comprise amino acid sequences including, but not limited to, GGSG (SEQ ID NO: 846), GGSGG (SEQ ID NO: 847), GSGSG (SEQ ID NO: 848), GSGGG (SEQ ID NO: 849), GGGSG (SEQ ID NO: 850), GSSSG (SEQ ID NO: 851), GGGGS (SEQ ID NO: 852) and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any desired element can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

NUCLEIC ACIDS AND RECOMBINANT EXPRESSION VECTORS

[00137] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure.

[00138] The present disclosure provides one or more nucleic acids comprising one or more of: a donor polynucleotide sequence, a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, a guide RNA, and a nucleotide sequence encoding a guide RNA (which can include two separate nucleotide sequences in the case of dual guide RNA format or which can include a single nucleotide sequence in the case of single guide RNA format).

[00139] The present disclosure provides a recombinant expression vector that comprises a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a recombinant expression vector that comprises a nucleotide sequence encoding a fusion polypeptide of the present disclosure. The present disclosure provides a recombinant expression vector that comprises: a) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; and b) a nucleotide sequence encoding a guide RNA(s). The present disclosure provides a recombinant expression vector that comprises: a) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; and b) a nucleotide sequence encoding a guide RNA(s). In some cases, the nucleotide sequence encoding the RNA-guided effector protein and/or the nucleotide sequence encoding the guide RNA is operably linked to a promoter that is operable in a cell type of choice (e.g., a

prokaryotic cell, a eukaryotic cell, a plant cell, an animal cell, a mammalian cell, a primate cell, a rodent cell, a human cell, etc.).

[00140] In some cases, a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure is codon optimized. This type of optimization can entail a mutation of an RNA-guided effector polypeptide-encoding nucleotide sequence to mimic the codon preferences of the intended host organism or cell while encoding the same protein. Thus, the codons can be changed, but the encoded protein remains unchanged. For example, if the intended target cell was a human cell, a human codon-optimized RNA-guided effector polypeptide-encoding nucleotide sequence could be used. As another non-limiting example, if the intended host cell were a mouse cell, then a mouse codon-optimized RNA-guided effector polypeptide-encoding nucleotide sequence could be generated. As another non-limiting example, if the intended host cell were a plant cell, then a plant codon-optimized RNA-guided effector polypeptide-encoding nucleotide sequence could be generated. As another non-limiting example, if the intended host cell were an insect cell, then an insect codon-optimized RNA-guided effector polypeptide-encoding nucleotide sequence could be generated.

[00141] The present disclosure provides one or more recombinant expression vectors that include (in different recombinant expression vectors in some cases, and in the same recombinant expression vector in some cases): (i) a nucleotide sequence of a donor template nucleic acid (where the donor template comprises a nucleotide sequence having homology to a target sequence of a target nucleic acid (e.g., a target genome)); (ii) a nucleotide sequence that encodes a guide RNA that hybridizes to a target sequence of the target locus of the targeted genome (e.g., a single or dual guide RNA) (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell); and (iii) a nucleotide sequence encoding RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell). The present disclosure provides one or more recombinant expression vectors that include (in different recombinant expression vectors in some cases, and in the same recombinant expression vector in some cases): (i) a nucleotide sequence of a donor template nucleic acid (where the donor template comprises a nucleotide sequence having homology to a target sequence of a target nucleic acid (e.g., a target genome)); and (ii) a nucleotide sequence that encodes a guide RNA that hybridizes to a target sequence of the target locus of the targeted genome (e.g., a single or dual guide RNA) (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell). The present disclosure provides one or more recombinant expression vectors that include (in different recombinant expression vectors in some cases, and in the same recombinant expression vector in some cases): (i) a nucleotide sequence that encodes a guide

RNA that hybridizes to a target sequence of the target locus of the targeted genome (e.g., a single or dual guide RNA) (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell); and (ii) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure (e.g., operably linked to a promoter that is operable in a target cell such as a eukaryotic cell).

[00142] Suitable expression vectors include viral expression vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., *Invest Ophthalmol Vis Sci* 35:2543 2549, 1994; Borrás et al., *Gene Ther* 6:515 524, 1999; Li and Davidson, *PNAS* 92:7700 7704, 1995; Sakamoto et al., *Gene Ther* 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (AAV) (see, e.g., Ali et al., *Hum Gene Ther* 9:81 86, 1998, Flannery et al., *PNAS* 94:6916 6921, 1997; Bennett et al., *Invest Ophthalmol Vis Sci* 38:2857 2863, 1997; Jomary et al., *Gene Ther* 4:683 690, 1997, Rolling et al., *Hum Gene Ther* 10:641 648, 1999; Ali et al., *Hum Mol Genet* 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., *J. Vir.* (1989) 63:3822-3828; Mendelson et al., *Virol.* (1988) 166:154-165; and Flotte et al., *PNAS* (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., *PNAS* 94:10319 23, 1997; Takahashi et al., *J Virol* 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, a lentivirus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like. In some cases, a recombinant expression vector of the present disclosure is a recombinant adeno-associated virus (AAV) vector. In some cases, a recombinant expression vector of the present disclosure is a recombinant lentivirus vector. In some cases, a recombinant expression vector of the present disclosure is a recombinant retroviral vector.

[00143] Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible promoters, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector.

[00144] In some embodiments, a nucleotide sequence encoding a guide RNA is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. In some embodiments, a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure is operably linked to a control element, e.g., a transcriptional control element, such as a promoter.

[00145] The transcriptional control element can be a promoter. In some cases, the promoter is a constitutively active promoter. In some cases, the promoter is a regulatable promoter. In some cases, the promoter is an inducible promoter. In some cases, the promoter is a tissue-specific

promoter. In some cases, the promoter is a cell type-specific promoter. In some cases, the transcriptional control element (e.g., the promoter) is functional in a targeted cell type or targeted cell population. For example, in some cases, the transcriptional control element can be functional in eukaryotic cells, e.g., hematopoietic stem cells (e.g., mobilized peripheral blood (mPB) CD34(+) cell, bone marrow (BM) CD34(+) cell, etc.).

[00146] Non-limiting examples of eukaryotic promoters (promoters functional in a eukaryotic cell) include EF1 α , those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Non-limiting examples of tissue specific promoters include Syn1 (neurons); GFAP (astrocytes); B29 (B cells); hAAT (liver); albumin (liver); MCR (muscle); SPB (lung), and the like. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression. The expression vector may also include nucleotide sequences encoding protein tags (e.g., 6xHis tag, hemagglutinin tag, fluorescent protein, etc.) that can be fused to the RNA-guided effector polypeptide of the present disclosure.

[00147] In some cases, a nucleotide sequence encoding a guide RNA and/or an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure is operably linked to an inducible promoter. In some cases, a nucleotide sequence encoding a guide RNA and/or an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure is operably linked to a constitutive promoter.

[00148] A promoter can be a constitutively active promoter (i.e., a promoter that is constitutively in an active/"ON" state), it may be an inducible promoter (i.e., a promoter whose state, active/"ON" or inactive/"OFF", is controlled by an external stimulus, e.g., the presence of a particular temperature, compound, or protein), it may be a spatially restricted promoter (i.e., transcriptional control element, enhancer, etc.)(e.g., tissue specific promoter, cell type specific promoter, etc.), and it may be a temporally restricted promoter (i.e., the promoter is in the "ON" state or "OFF" state during specific stages of embryonic development or during specific stages of a biological process, e.g., hair follicle cycle in mice).

[00149] Suitable promoters can be derived from viruses and can therefore be referred to as viral promoters, or they can be derived from any organism, including prokaryotic or eukaryotic organisms. Suitable promoters can be used to drive expression by any RNA polymerase (e.g., pol I, pol II, pol III). Exemplary promoters include, but are not limited to the SV40 early promoter, mouse mammary tumor virus long terminal repeat (LTR) promoter; adenovirus major late

promoter (Ad MLP); a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), a rous sarcoma virus (RSV) promoter, a human U6 small nuclear promoter (U6) (Miyagishi et al., Nature Biotechnology 20, 497 - 500 (2002)), an enhanced U6 promoter (e.g., Xia et al., Nucleic Acids Res. 2003 Sep 1;31(17)), a human H1 promoter (H1), and the like.

[00150] In some cases, a nucleotide sequence encoding a guide RNA is operably linked to (under the control of) a promoter operable in a eukaryotic cell (e.g., a U6 promoter, an enhanced U6 promoter, an H1 promoter, and the like). As would be understood by one of ordinary skill in the art, when expressing an RNA (e.g., a guide RNA) from a nucleic acid (e.g., an expression vector) using a U6 promoter (e.g., in a eukaryotic cell), or another PolIII promoter, the RNA may need to be mutated if there are several Ts in a row (coding for Us in the RNA). This is because a string of Ts (e.g., 5 Ts) in DNA can act as a terminator for polymerase III (PolIII). Thus, in order to ensure transcription of a guide RNA (e.g., the activator portion and/or targeter portion, in dual guide or single guide format) in a eukaryotic cell it may sometimes be necessary to modify the sequence encoding the guide RNA to eliminate runs of Ts. In some cases, a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure is operably linked to a promoter operable in a eukaryotic cell (e.g., a CMV promoter, an EF1 α promoter, an estrogen receptor-regulated promoter, and the like).

[00151] Examples of inducible promoters include, but are not limited to T7 RNA polymerase promoter, T3 RNA polymerase promoter, Isopropyl-beta-D-thiogalactopyranoside (IPTG)-regulated promoter, lactose induced promoter, heat shock promoter, Tetracycline-regulated promoter, Steroid-regulated promoter, Metal-regulated promoter, estrogen receptor-regulated promoter, etc. Inducible promoters can therefore be regulated by molecules including, but not limited to, doxycycline; estrogen and/or an estrogen analog; IPTG; etc.

[00152] Inducible promoters suitable for use include any inducible promoter described herein or known to one of ordinary skill in the art. Examples of inducible promoters include, without limitation, chemically/biochemically-regulated and physically-regulated promoters such as alcohol-regulated promoters, rapamycin-regulated promoters, tetracycline-regulated promoters (e.g., anhydrotetracycline (aTc)-responsive promoters and other tetracycline-responsive promoter systems, which include a tetracycline repressor protein (tetR), a tetracycline operator sequence (tetO) and a tetracycline transactivator fusion protein (tTA)), steroid-regulated promoters (e.g., promoters based on the rat glucocorticoid receptor, human estrogen receptor, moth ecdysone receptors, and promoters from the steroid/retinoid/thyroid receptor superfamily), metal-regulated promoters (e.g., promoters derived from metallothionein (proteins that bind and

sequester metal ions) genes from yeast, mouse and human), pathogenesis-regulated promoters (e.g., induced by salicylic acid, ethylene or benzothiadiazole (BTH)), temperature/heat-inducible promoters (e.g., heat shock promoters), and light-regulated promoters (e.g., light responsive promoters from plant cells).

[00153] In some cases, the promoter is a spatially restricted promoter (i.e., cell type specific promoter, tissue specific promoter, etc.) such that in a multi-cellular organism, the promoter is active (i.e., “ON”) in a subset of specific cells. Spatially restricted promoters may also be referred to as enhancers, transcriptional control elements, control sequences, etc. Any convenient spatially restricted promoter may be used as long as the promoter is functional in the targeted host cell (e.g., eukaryotic cell; prokaryotic cell).

[00154] In some cases, the promoter is a reversible promoter. Suitable reversible promoters, including reversible inducible promoters are known in the art. Such reversible promoters may be isolated and derived from many organisms, e.g., eukaryotes and prokaryotes. Modification of reversible promoters derived from a first organism for use in a second organism, e.g., a first prokaryote and a second a eukaryote, a first eukaryote and a second a prokaryote, etc., is well known in the art. Such reversible promoters, and systems based on such reversible promoters but also comprising additional control proteins, include, but are not limited to, alcohol regulated promoters (e.g., alcohol dehydrogenase I (alcA) gene promoter, promoters responsive to alcohol transactivator proteins (AlcR), etc.), tetracycline regulated promoters, (e.g., promoter systems including TetActivators, TetON, TetOFF, etc.), steroid regulated promoters (e.g., rat glucocorticoid receptor promoter systems, human estrogen receptor promoter systems, retinoid promoter systems, thyroid promoter systems, ecdysone promoter systems, mifepristone promoter systems, etc.), metal regulated promoters (e.g., metallothionein promoter systems, etc.), pathogenesis-related regulated promoters (e.g., salicylic acid regulated promoters, ethylene regulated promoters, benzothiadiazole regulated promoters, etc.), temperature regulated promoters (e.g., heat shock inducible promoters (e.g., HSP-70, HSP-90, soybean heat shock promoter, etc.), light regulated promoters, synthetic inducible promoters, and the like.

[00155] Methods of introducing a nucleic acid (e.g., a nucleic acid comprising a donor polynucleotide sequence, one or more nucleic acids encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure and/or a guide RNA, and the like) into a host cell are known in the art, and any convenient method can be used to introduce a nucleic acid (e.g., an expression construct) into a cell. Suitable methods include e.g., viral infection, transfection, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated

transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct microinjection, nanoparticle-mediated nucleic acid delivery, and the like.

[00156] Introducing the recombinant expression vector into cells can occur in any culture media and under any culture conditions that promote the survival of the cells. Introducing the recombinant expression vector into a target cell can be carried out *in vivo* or *ex vivo*. Introducing the recombinant expression vector into a target cell can be carried out *in vitro*.

[00157] In some embodiments, an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure can be provided as RNA. The RNA can be provided by direct chemical synthesis or may be transcribed *in vitro* from a DNA (e.g., encoding the RNA-guided effector polypeptide of the present disclosure or the fusion polypeptide of the present disclosure). Once synthesized, the RNA may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc.).

[00158] Nucleic acids may be provided to the cells using well-developed transfection techniques; see, e.g. Angel and Yanik (2010) PLoS ONE 5(7): e11756, and the commercially available TransMessenger® reagents from Qiagen, Stemfect™ RNA Transfection Kit from Stemgent, and TransIT®-mRNA Transfection Kit from Mirus Bio LLC. See also Beumer et al. (2008) PNAS 105(50):19821-19826.

[00159] Vectors may be provided directly to a target host cell. In other words, the cells are contacted with vectors comprising the subject nucleic acids (e.g., recombinant expression vectors having the donor template sequence and encoding the guide RNA; recombinant expression vectors encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure; etc.) such that the vectors are taken up by the cells. Methods for contacting cells with nucleic acid vectors that are plasmids, include electroporation, calcium chloride transfection, microinjection, and lipofection are well known in the art. For viral vector delivery, cells can be contacted with viral particles comprising the subject viral expression vectors.

[00160] Retroviruses, for example, lentiviruses, are suitable for use in methods of the present disclosure. Commonly used retroviral vectors are “defective”, i.e. unable to produce viral proteins required for productive infection. Rather, replication of the vector requires growth in a packaging cell line. To generate viral particles comprising nucleic acids of interest, the retroviral nucleic acids comprising the nucleic acid are packaged into viral capsids by a packaging cell line. Different packaging cell lines provide a different envelope protein (ecotropic, amphotropic or xenotropic) to be incorporated into the capsid, this envelope protein determining the

specificity of the viral particle for the cells (ecotropic for murine and rat; amphotropic for most mammalian cell types including human, dog and mouse; and xenotropic for most mammalian cell types except murine cells). The appropriate packaging cell line may be used to ensure that the cells are targeted by the packaged viral particles. Methods of introducing subject vector expression vectors into packaging cell lines and of collecting the viral particles that are generated by the packaging lines are well known in the art. Nucleic acids can also be introduced by direct micro-injection (e.g., injection of RNA).

[00161] Vectors used for providing the nucleic acids encoding a guide RNA and/or an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure to a target host cell can include suitable promoters for driving the expression, that is, transcriptional activation, of the nucleic acid of interest. In other words, in some cases, the nucleic acid of interest will be operably linked to a promoter. This may include ubiquitously acting promoters, for example, the CMV- β -actin promoter, or inducible promoters, such as promoters that are active in particular cell populations or that respond to the presence of drugs such as tetracycline. By transcriptional activation, it is intended that transcription will be increased above basal levels in the target cell by 10 fold, by 100 fold, more usually by 1000 fold. In addition, vectors used for providing a nucleic acid encoding a guide RNA and/or an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure to a cell may include nucleic acid sequences that encode for selectable markers in the target cells, so as to identify cells that have taken up the guide RNA and/or the RNA-guided effector polypeptide of the present disclosure or the fusion polypeptide of the present disclosure.

[00162] A nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure, is in some cases an RNA. Thus, an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure can be introduced into cells as RNA. Methods of introducing RNA into cells are known in the art and may include, for example, direct injection, transfection, incorporation of RNA into lipid nanoparticles, or any other method used for the introduction of DNA. An RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure protein may instead be provided to cells as a polypeptide. Such a polypeptide may optionally be fused to a polypeptide domain that increases solubility of the product. The domain may be linked to the polypeptide through a defined protease cleavage site, e.g. a TEV sequence, which is cleaved by TEV protease. The linker may also include one or more flexible sequences, e.g. from 1 to 10 glycine residues. In some embodiments, the cleavage of the fusion protein is performed in a buffer that maintains solubility of the product, e.g. in the presence of from 0.5 to 2 M urea, in the presence of polypeptides and/or polynucleotides that

increase solubility, and the like. Domains of interest include endosomolytic domains, e.g. influenza HA domain; and other polypeptides that aid in production, e.g. IF2 domain, GST domain, GRPE domain, and the like. The polypeptide may be formulated for improved stability. For example, the peptides may be PEGylated, where the polyethyleneoxy group provides for enhanced lifetime in the blood stream.

[00163] Additionally or alternatively, an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure may be fused to a polypeptide permeant domain to promote uptake by the cell. A number of permeant domains are known in the art and may be used in the non-integrating polypeptides of the present disclosure, including peptides, peptidomimetics, and non-peptide carriers. For example, a permeant peptide may be derived from the third alpha helix of *Drosophila melanogaster* transcription factor Antennapedia, referred to as penetratin, which comprises the amino acid sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 853). As another example, the permeant peptide comprises the HIV-1 tat basic region amino acid sequence, which may include, for example, amino acids 49-57 of naturally-occurring tat protein. Other permeant domains include poly-arginine motifs, for example, the region of amino acids 34-56 of HIV-1 rev protein, nona-arginine, octa-arginine, and the like. (See, for example, Futaki et al. (2003) *Curr Protein Pept Sci.* 2003 Apr; 4(2): 87-9 and 446; and Wender et al. (2000) *Proc. Natl. Acad. Sci. U.S.A* 2000 Nov. 21; 97(24):13003-8; published U.S. Patent applications 20030220334; 20030083256; 20030032593; and 20030022831, herein specifically incorporated by reference for the teachings of translocation peptides and peptoids). The nona-arginine (R9) sequence is one of the more efficient PTDs that have been characterized (Wender et al. 2000; Uemura et al. 2002). The site at which the fusion is made may be selected in order to optimize the biological activity, secretion or binding characteristics of the polypeptide. The optimal site will be determined by routine experimentation.

[00164] An RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure may be produced *in vitro* or by eukaryotic cells or by prokaryotic cells, and it may be further processed by unfolding, e.g. heat denaturation, dithiothreitol reduction, etc. and may be further refolded, using methods known in the art.

[00165] Modifications of interest that do not alter primary sequence include chemical derivatization of polypeptides, e.g., acylation, acetylation, carboxylation, amidation, etc. Also included are modifications of glycosylation, e.g. those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; e.g. by exposing the polypeptide to enzymes which affect glycosylation, such as mammalian

glycosylating or deglycosylating enzymes. Also embraced are sequences that have phosphorylated amino acid residues, e.g. phosphotyrosine, phosphoserine, or phosphothreonine.

[00166] Also suitable for inclusion in embodiments of the present disclosure are nucleic acids (e.g., encoding a guide RNA, encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure) that have been modified using ordinary molecular biological techniques and synthetic chemistry so as to improve their resistance to proteolytic degradation, to change the target sequence specificity, to optimize solubility properties, to alter protein activity (e.g., transcription modulatory activity, enzymatic activity, etc.) or to render them more suitable. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, e.g. D-amino acids or non-naturally occurring synthetic amino acids. D-amino acids may be substituted for some or all of the amino acid residues.

[00167] An RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure may be prepared by *in vitro* synthesis, using conventional methods as known in the art. Various commercial synthetic apparatuses are available, for example, automated synthesizers by Applied Biosystems, Inc., Beckman, etc. By using synthesizers, naturally occurring amino acids may be substituted with unnatural amino acids. The particular sequence and the manner of preparation will be determined by convenience, economics, purity required, and the like.

[00168] If desired, various groups may be introduced into the peptide during synthesis or during expression, which allow for linking to other molecules or to a surface. Thus cysteines can be used to make thioethers, histidines for linking to a metal ion complex, carboxyl groups for forming amides or esters, amino groups for forming amides, and the like.

[00169] An RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure may also be isolated and purified in accordance with conventional methods of recombinant synthesis. A lysate may be prepared of the expression host and the lysate purified using high performance liquid chromatography (HPLC), exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. For the most part, the compositions which are used will comprise 20% or more by weight of the desired product, e.g. 75% or more by weight, 95% or more by weight, or 99.5% or more by weight, in relation to contaminants related to the method of preparation of the product and its purification. Usually, the percentages will be based upon total protein. Thus, in some cases, an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure is at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure (e.g., free of contaminants, proteins other than the RNA-guided

effector polypeptide of the present disclosure or the fusion polypeptide of the present disclosure or other macromolecules, etc.).

[00170] To induce cleavage or any desired modification to a target nucleic acid (e.g., genomic DNA), or any desired modification to a polypeptide associated with target nucleic acid, a guide RNA and/or an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure and/or the donor template sequence, whether they be introduced as nucleic acids or polypeptides, are provided to the cells for about 30 minutes to about 24 hours, e.g., 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 12 hours, 16 hours, 18 hours, 20 hours, or any other period from about 30 minutes to about 24 hours, which may be repeated with a frequency of about every day to about every 4 days, e.g., every 1.5 days, every 2 days, every 3 days, or any other frequency from about every day to about every four days. The agent(s) may be provided to the subject cells one or more times, e.g. one time, twice, three times, or more than three times, and the cells allowed to incubate with the agent(s) for some amount of time following each contacting event e.g. 16-24 hours, after which time the media is replaced with fresh media and the cells are cultured further.

[00171] In cases in which two or more different targeting complexes are provided to the cell (e.g., two different guide RNAs that are complementary to different sequences within the same or different target nucleic acid), the complexes may be provided simultaneously (e.g. as two polypeptides and/or nucleic acids), or delivered simultaneously. Alternatively, they may be provided consecutively, e.g. the targeting complex being provided first, followed by the second targeting complex, etc. or vice versa.

[00172] To improve the delivery of a DNA vector into a target cell, the DNA can be protected from damage and its entry into the cell facilitated, for example, by using lipoplexes and polyplexes. Thus, in some cases, a nucleic acid of the present disclosure (e.g., a recombinant expression vector of the present disclosure) can be covered with lipids in an organized structure like a micelle or a liposome. When the organized structure is complexed with DNA it is called a lipoplex. There are three types of lipids, anionic (negatively-charged), neutral, or cationic (positively-charged). Lipoplexes that utilize cationic lipids have proven utility for gene transfer. Cationic lipids, due to their positive charge, naturally complex with the negatively charged DNA. Also as a result of their charge, they interact with the cell membrane. Endocytosis of the lipoplex then occurs, and the DNA is released into the cytoplasm. The cationic lipids also protect against degradation of the DNA by the cell.

[00173] Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions. One large difference between the methods of action of polyplexes and lipoplexes is that polyplexes cannot release

their DNA load into the cytoplasm, so to this end, co-transfection with endosome-lytic agents (to lyse the endosome that is made during endocytosis) such as inactivated adenovirus must occur. However, this is not always the case; polymers such as polyethylenimine have their own method of endosome disruption as does chitosan and trimethylchitosan.

[00174] Dendrimers, a highly branched macromolecule with a spherical shape, may be also be used to genetically modify stem cells. The surface of the dendrimer particle may be functionalized to alter its properties. In particular, it is possible to construct a cationic dendrimer (i.e., one with a positive surface charge). When in the presence of genetic material such as a DNA plasmid, charge complementarity leads to a temporary association of the nucleic acid with the cationic dendrimer. On reaching its destination, the dendrimer-nucleic acid complex can be taken up into a cell by endocytosis.

[00175] In some cases, a nucleic acid of the disclosure (e.g., an expression vector) includes an insertion site for a guide sequence of interest. For example, a nucleic acid can include an insertion site for a guide sequence of interest, where the insertion site is immediately adjacent to a nucleotide sequence encoding the portion of a guide RNA that does not change when the guide sequence is changed to hybridized to a desired target sequence (e.g., sequences that contribute to the effector polypeptide binding aspect of the guide RNA, e.g., the sequences that contribute to the dsRNA duplex(es) of the guide RNA – this portion of the guide RNA can also be referred to as the ‘scaffold’ or ‘constant region’ of the guide RNA). Thus, in some cases, a subject nucleic acid (e.g., an expression vector) includes a nucleotide sequence encoding a guide RNA, except that the portion encoding the guide sequence portion of the guide RNA is an insertion sequence (an insertion site). An insertion site is any nucleotide sequence used for the insertion of a desired sequence. “Insertion sites” for use with various technologies are known to those of ordinary skill in the art and any convenient insertion site can be used. An insertion site can be for any method for manipulating nucleic acid sequences. For example, in some cases the insertion site is a multiple cloning site (MCS) (e.g., a site including one or more restriction enzyme recognition sequences), a site for ligation independent cloning, a site for recombination based cloning (e.g., recombination based on att sites), a nucleotide sequence recognized by a CRISPR/Cas (e.g. Cas9) based technology, a Cre site, a FLP site, and the like.

[00176] An insertion site can be any desirable length, and can depend on the type of insertion site (e.g., can depend on whether (and how many) the site includes one or more restriction enzyme recognition sequences, whether the site includes a target site for a CRISPR/Cas protein, etc.). In some cases, an insertion site of a subject nucleic acid is 3 or more nucleotides (nt) in length (e.g., 5 or more, 8 or more, 10 or more, 15 or more, 17 or more, 18 or more, 19 or more, 20 or more or 25 or more, or 30 or more nt in length). In some cases, the length of an insertion site of a subject

nucleic acid has a length in a range of from 2 to 50 nucleotides (nt) (e.g., from 2 to 40 nt, from 2 to 30 nt, from 2 to 25 nt, from 2 to 20 nt, from 5 to 50 nt, from 5 to 40 nt, from 5 to 30 nt, from 5 to 25 nt, from 5 to 20 nt, from 10 to 50 nt, from 10 to 40 nt, from 10 to 30 nt, from 10 to 25 nt, from 10 to 20 nt, from 17 to 50 nt, from 17 to 40 nt, from 17 to 30 nt, from 17 to 25 nt). In some cases, the length of an insertion site of a subject nucleic acid has a length in a range of from 5 to 40 nt.

[00177] In some cases, a nucleic acid of the present disclosure (e.g., an expression vector) comprises: i) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; and ii) an insertion site for a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest. The insertion site can be located relative to the nucleotide sequence encoding the RNA-guided effector polypeptide, such that, after insertion of a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest, the resulting fusion polypeptide would comprise, in order from N-terminus to C-terminus: i) the RNA-guided effector polypeptide; and ii) the fusion partner. The insertion site can be located relative to the nucleotide sequence encoding the RNA-guided effector polypeptide, such that, after insertion of a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest, the resulting fusion polypeptide would comprise, in order from N-terminus to C-terminus: i) the fusion partner; and ii) the RNA-guided effector polypeptide. An insertion site is any nucleotide sequence used for the insertion of a desired sequence. "Insertion sites" for use with various technologies are known to those of ordinary skill in the art and any convenient insertion site can be used. An insertion site can be for any method for manipulating nucleic acid sequences. For example, in some cases the insertion site is a multiple cloning site (MCS) (e.g., a site including one or more restriction enzyme recognition sequences), a site for ligation independent cloning, a site for recombination based cloning (e.g., recombination based on att sites), a nucleotide sequence recognized by a CRISPR/Cas (e.g. Cas9) based technology, a Cre site, a FLP site, and the like.

Guide RNA

[00178] A nucleic acid that binds to a Cas9 protein and targets the complex to a specific location within a target nucleic acid is referred to herein as a "Cas9 guide RNA" or, simply "a guide RNA." A Cas9 guide RNA binds to an RNA-guided effector polypeptide of the present disclosure, forming an RNA-guided effector polypeptide/guide RNA complex, and targets the complex to a specific location (nucleotide sequence) within a target nucleic acid. A Cas9 guide RNA binds to a fusion polypeptide of the present disclosure, forming a fusion polypeptide/guide RNA complex, and targets the complex to a specific location (nucleotide sequence) within a target nucleic acid. A guide RNA that binds to a Cas9 polypeptide (e.g., a Spy Cas9 polypeptide

as depicted in FIG. 1) also binds to an RNA-guided effector polypeptide of the present disclosure, and to a fusion polypeptide of the present disclosure.

[00179] A Cas9 guide RNA (can be said to include two segments, a first segment (referred to herein as a “targeting segment”); and a second segment (referred to herein as a “protein-binding segment”). By “segment” it is meant a segment/section/region of a molecule, e.g., a contiguous stretch of nucleotides in a nucleic acid molecule. A segment can also mean a region/section of a complex such that a segment may comprise regions of more than one molecule.

[00180] The first segment (targeting segment) of a Cas9 guide RNA includes a nucleotide sequence (a guide sequence) that is complementary to (and therefore hybridizes with) a specific sequence (a target site) within a target nucleic acid (e.g., a target ssRNA, a target ssDNA, the complementary strand of a double stranded target DNA, etc.). The protein-binding segment (or “protein-binding sequence”) interacts with (binds to) a Cas9 polypeptide, an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure. The protein-binding segment of a subject Cas9 guide RNA includes two complementary stretches of nucleotides that hybridize to one another to form a double stranded RNA duplex (dsRNA duplex). Site-specific binding and/or cleavage of a target nucleic acid (e.g., genomic DNA) can occur at locations (e.g., target sequence of a target locus) determined by base-pairing complementarity between the Cas9 guide RNA (the guide sequence of the Cas9 guide RNA) and the target nucleic acid.

[00181] A Cas9 guide RNA and a Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure) form a complex (e.g., bind via non-covalent interactions). The Cas9 guide RNA provides target specificity to the complex by including a targeting segment, which includes a guide sequence (a nucleotide sequence that is complementary to a sequence of a target nucleic acid). The Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure) of the complex provides the site-specific activity (e.g., binding activity provided by an RNA-guided effector polypeptide; binding activity provided by the RNA-guided effector portion of a fusion polypeptide of the present disclosure; or an activity provided by the fusion partner of a fusion polypeptide of the present disclosure). In other words, the Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure) is guided to a target nucleic acid sequence (e.g. a target sequence in a chromosomal nucleic acid, e.g., a chromosome; a target sequence in an extrachromosomal nucleic acid, e.g. an episomal nucleic acid, a minicircle, an ssRNA, an ssDNA, etc.; a target sequence in a mitochondrial nucleic acid; a target sequence in a chloroplast nucleic acid; a target sequence in a plasmid; a target sequence in a viral nucleic acid; etc.) by virtue of its association with the Cas9 guide RNA.

- [00182]** The “guide sequence” also referred to as the “targeting sequence” of a guide RNA can be modified so that the guide RNA can target a Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure) to any desired sequence of any desired target nucleic acid, with the exception that the protospacer adjacent motif (PAM) sequence can be taken into account. Thus, for example, a guide RNA can have a targeting segment with a sequence (a guide sequence) that has complementarity with (e.g., can hybridize to) a sequence in a nucleic acid in a eukaryotic cell, e.g., a viral nucleic acid, a eukaryotic nucleic acid (e.g., a eukaryotic chromosome, chromosomal sequence, a eukaryotic RNA, etc.), and the like.
- [00183]** In some cases, a Cas9 guide RNA includes two separate nucleic acid molecules: an “activator” and a “targeter” and is referred to herein as a “dual Cas9 guide RNA”, a “double-molecule Cas9 guide RNA”, or a “two-molecule Cas9 guide RNA” a “dual guide RNA”, or a “dgRNA.” In some cases, the activator and targeter are covalently linked to one another (e.g., via intervening nucleotides) and the guide RNA is referred to as a “single guide RNA”, a “Cas9 single guide RNA”, a “single-molecule Cas9 guide RNA,” or a “one-molecule Cas9 guide RNA”, or simply “sgRNA.”
- [00184]** A guide RNA comprises a crRNA-like (“CRISPR RNA” / “targeter” / “crRNA” / “crRNA repeat”) molecule and a corresponding tracrRNA-like (“trans-acting CRISPR RNA” / “activator” / “tracrRNA”) molecule. A crRNA-like molecule (targeter) comprises both the targeting segment (single stranded) of the guide RNA and a stretch (“duplex-forming segment”) of nucleotides that forms one half of the dsRNA duplex of the protein-binding segment of the guide RNA. A corresponding tracrRNA-like molecule (activator / tracrRNA) comprises a stretch of nucleotides (duplex-forming segment) that forms the other half of the dsRNA duplex of the protein-binding segment of the guide nucleic acid. In other words, a stretch of nucleotides of a crRNA-like molecule are complementary to and hybridize with a stretch of nucleotides of a tracrRNA-like molecule to form the dsRNA duplex of the protein-binding domain of the Cas9 guide RNA. As such, each targeter molecule can be said to have a corresponding activator molecule (which has a region that hybridizes with the targeter). The targeter molecule additionally provides the targeting segment. Thus, a targeter and an activator molecule (as a corresponding pair) hybridize to form a Cas9 guide RNA. The exact sequence of a given crRNA or tracrRNA molecule is characteristic of the species in which the RNA molecules are found. A subject dual Cas9 guide RNA can include any corresponding activator and targeter pair.
- [00185]** The term “activator” or “activator RNA” is used herein to mean a tracrRNA-like molecule (tracrRNA : “trans-acting CRISPR RNA”) of a Cas9 dual guide RNA (and therefore of a Cas9 single guide RNA when the “activator” and the “targeter” are linked together by, e.g.,

intervening nucleotides). Thus, for example, a Cas9 guide RNA (dgRNA or sgRNA) comprises an activator sequence (e.g., a tracrRNA sequence). A tracr molecule (a tracrRNA) is a naturally existing molecule that hybridizes with a CRISPR RNA molecule (a crRNA) to form a Cas9 dual guide RNA. The term “activator” is used herein to encompass naturally existing tracrRNAs, but also to encompass tracrRNAs with modifications (e.g., truncations, sequence variations, base modifications, backbone modifications, linkage modifications, etc.) where the activator retains at least one function of a tracrRNA (e.g., contributes to the dsRNA duplex to which Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure) binds). In some cases the activator provides one or more stem loops that can interact with Cas9 protein (or an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure). An activator can be referred to as having a tracr sequence (tracrRNA sequence) and in some cases is a tracrRNA, but the term “activator” is not limited to naturally existing tracrRNAs.

[00186] The term “targeter” or “targeter RNA” is used herein to refer to a crRNA-like molecule (crRNA: “CRISPR RNA”) of a Cas9 dual guide RNA (and therefore of a Cas9 single guide RNA when the “activator” and the “targeter” are linked together, e.g., by intervening nucleotides). Thus, for example, a Cas9 guide RNA (dgRNA or sgRNA) comprises a targeting segment (which includes nucleotides that hybridize with (are complementary to) a target nucleic acid, and a duplex-forming segment (e.g., a duplex forming segment of a crRNA, which can also be referred to as a crRNA repeat). Because the sequence of a targeting segment (the segment that hybridizes with a target sequence of a target nucleic acid) of a targeter is modified by a user to hybridize with a desired target nucleic acid, the sequence of a targeter will often be a non-naturally occurring sequence. However, the duplex-forming segment of a targeter (described in more detail below), which hybridizes with the duplex-forming segment of an activator, can include a naturally existing sequence (e.g., can include the sequence of a duplex-forming segment of a naturally existing crRNA, which can also be referred to as a crRNA repeat). Thus, the term targeter is used herein to distinguish from naturally occurring crRNAs, despite the fact that part of a targeter (e.g., the duplex-forming segment) often includes a naturally occurring sequence from a crRNA. However, the term “targeter” encompasses naturally occurring crRNAs.

[00187] A Cas9 guide RNA can also be said to include 3 parts: (i) a targeting sequence (a nucleotide sequence that hybridizes with a sequence of the target nucleic acid); (ii) an activator sequence (as described above)(in some cases, referred to as a tracr sequence); and (iii) a sequence that hybridizes to at least a portion of the activator sequence to form a double stranded duplex. A targeter has (i) and (iii); while an activator has (ii).

[00188] A Cas9 guide RNA (e.g. a dual guide RNA or a single guide RNA) can be comprised of any corresponding activator and targeter pair. In some cases, the duplex forming segments can be swapped between the activator and the targeter. In other words, in some cases, the targeter includes a sequence of nucleotides from a duplex forming segment of a tracrRNA (which sequence would normally be part of an activator) while the activator includes a sequence of nucleotides from a duplex forming segment of a crRNA (which sequence would normally be part of a targeter).

[00189] As noted above, a targeter comprises both the targeting segment (single stranded) of the Cas9 guide RNA and a stretch (“duplex-forming segment”) of nucleotides that forms one half of the dsRNA duplex of the protein-binding segment of the Cas9 guide RNA. A corresponding tracrRNA-like molecule (activator) comprises a stretch of nucleotides (a duplex-forming segment) that forms the other half of the dsRNA duplex of the protein-binding segment of the Cas9 guide RNA. In other words, a stretch of nucleotides of the targeter is complementary to and hybridizes with a stretch of nucleotides of the activator to form the dsRNA duplex of the protein-binding segment of a Cas9 guide RNA. As such, each targeter can be said to have a corresponding activator (which has a region that hybridizes with the targeter). The targeter molecule additionally provides the targeting segment. Thus, a targeter and an activator (as a corresponding pair) hybridize to form a Cas9 guide RNA. The particular sequence of a given naturally existing crRNA or tracrRNA molecule is characteristic of the species in which the RNA molecules are found. Examples of suitable activator and targeter are well known in the art.

Targeting segment of a Cas9 guide RNA

[00190] The first segment of a subject guide nucleic acid includes a guide sequence (i.e., a targeting sequence)(a nucleotide sequence that is complementary to a sequence (a target site) in a target nucleic acid). In other words, the targeting segment of a subject guide nucleic acid can interact with a target nucleic acid (e.g., double stranded DNA (dsDNA)) in a sequence-specific manner via hybridization (i.e., base pairing). As such, the nucleotide sequence of the targeting segment may vary (depending on the target) and can determine the location within the target nucleic acid that the Cas9 guide RNA and the target nucleic acid will interact. The targeting segment of a Cas9 guide RNA can be modified (e.g., by genetic engineering)/designed to hybridize to any desired sequence (target site) within a target nucleic acid (e.g., a eukaryotic target nucleic acid such as genomic DNA).

[00191] The targeting segment can have a length of 7 or more nucleotides (nt) (e.g., 8 or more, 9 or more, 10 or more, 12 or more, 15 or more, 20 or more, 25 or more, 30 or more, or 40 or more nucleotides). In some cases, the targeting segment can have a length of from 7 to 100 nucleotides (nt) (e.g., from 7 to 80 nt, from 7 to 60 nt, from 7 to 40 nt, from 7 to 30 nt, from 7 to 25 nt, from

7 to 22 nt, from 7 to 20 nt, from 7 to 18 nt, from 8 to 80 nt, from 8 to 60 nt, from 8 to 40 nt, from 8 to 30 nt, from 8 to 25 nt, from 8 to 22 nt, from 8 to 20 nt, from 8 to 18 nt, from 10 to 100 nt, from 10 to 80 nt, from 10 to 60 nt, from 10 to 40 nt, from 10 to 30 nt, from 10 to 25 nt, from 10 to 22 nt, from 10 to 20 nt, from 10 to 18 nt, from 12 to 100 nt, from 12 to 80 nt, from 12 to 60 nt, from 12 to 40 nt, from 12 to 30 nt, from 12 to 25 nt, from 12 to 22 nt, from 12 to 20 nt, from 12 to 18 nt, from 14 to 100 nt, from 14 to 80 nt, from 14 to 60 nt, from 14 to 40 nt, from 14 to 30 nt, from 14 to 25 nt, from 14 to 22 nt, from 14 to 20 nt, from 14 to 18 nt, from 16 to 100 nt, from 16 to 80 nt, from 16 to 60 nt, from 16 to 40 nt, from 16 to 30 nt, from 16 to 25 nt, from 16 to 22 nt, from 16 to 20 nt, from 16 to 18 nt, from 18 to 100 nt, from 18 to 80 nt, from 18 to 60 nt, from 18 to 40 nt, from 18 to 30 nt, from 18 to 25 nt, from 18 to 22 nt, or from 18 to 20 nt).

[00192] The nucleotide sequence (the targeting sequence) of the targeting segment that is complementary to a nucleotide sequence (target site) of the target nucleic acid can have a length of 10 nt or more. For example, the targeting sequence of the targeting segment that is complementary to a target site of the target nucleic acid can have a length of 12 nt or more, 15 nt or more, 18 nt or more, 19 nt or more, or 20 nt or more. In some cases, the nucleotide sequence (the targeting sequence) of the targeting segment that is complementary to a nucleotide sequence (target site) of the target nucleic acid has a length of 12 nt or more. In some cases, the nucleotide sequence (the targeting sequence) of the targeting segment that is complementary to a nucleotide sequence (target site) of the target nucleic acid has a length of 18 nt or more.

[00193] For example, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid can have a length of from 10 to 100 nucleotides (nt) (e.g., from 10 to 90 nt, from 10 to 75 nt, from 10 to 60 nt, from 10 to 50 nt, from 10 to 35 nt, from 10 to 30 nt, from 10 to 25 nt, from 10 to 22 nt, from 10 to 20 nt, from 12 to 100 nt, from 12 to 90 nt, from 12 to 75 nt, from 12 to 60 nt, from 12 to 50 nt, from 12 to 35 nt, from 12 to 30 nt, from 12 to 25 nt, from 12 to 22 nt, from 12 to 20 nt, from 15 to 100 nt, from 15 to 90 nt, from 15 to 75 nt, from 15 to 60 nt, from 15 to 50 nt, from 15 to 35 nt, from 15 to 30 nt, from 15 to 25 nt, from 15 to 22 nt, from 15 to 20 nt, from 17 to 100 nt, from 17 to 90 nt, from 17 to 75 nt, from 17 to 60 nt, from 17 to 50 nt, from 17 to 35 nt, from 17 to 30 nt, from 17 to 25 nt, from 17 to 22 nt, from 17 to 20 nt, from 18 to 100 nt, from 18 to 90 nt, from 18 to 75 nt, from 18 to 60 nt, from 18 to 50 nt, from 18 to 35 nt, from 18 to 30 nt, from 18 to 25 nt, from 18 to 22 nt, or from 18 to 20 nt). In some cases, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid has a length of from 15 nt to 30 nt. In some cases, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid has a length of from 15 nt to 25 nt. In some cases, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid has a

length of from 18 nt to 30 nt. In some cases, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid has a length of from 18 nt to 25 nt. In some cases, the targeting sequence of the targeting segment that is complementary to a target sequence of the target nucleic acid has a length of from 18 nt to 22 nt. In some cases, the targeting sequence of the targeting segment that is complementary to a target site of the target nucleic acid is 20 nucleotides in length. In some cases, the targeting sequence of the targeting segment that is complementary to a target site of the target nucleic acid is 19 nucleotides in length.

[00194] The percent complementarity between the targeting sequence (guide sequence) of the targeting segment and the target site of the target nucleic acid can be 60% or more (e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 97% or more, 98% or more, 99% or more, or 100%). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the seven contiguous 5'-most nucleotides of the target site of the target nucleic acid. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 60% or more over about 20 contiguous nucleotides. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the fourteen contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 14 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the seven contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 20 nucleotides in length.

[00195] In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 7 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 8 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 9 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can

be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 10 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 17 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 18 contiguous 5'-most nucleotides of the target site of the target nucleic acid (which can be complementary to the 3'-most nucleotides of the targeting sequence of the Cas9 guide RNA). In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 60% or more (e.g., e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 97% or more, 98% or more, 99% or more, or 100%) over about 20 contiguous nucleotides.

[00196] In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 7 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 7 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 8 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 8 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 9 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 9 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 10 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 10 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 11 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the

remainder. In such a case, the targeting sequence can be considered to be 11 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 12 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 12 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 13 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 13 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 14 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 14 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 17 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 17 nucleotides in length. In some cases, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the 18 contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 18 nucleotides in length.

[00197] Examples of various Cas9 proteins and Cas9 guide RNAs (as well as information regarding requirements related to protospacer adjacent motif (PAM) sequences present in targeted nucleic acids) can be found in the art, for example, see Jinek et al., *Science*. 2012 Aug 17;337(6096):816-21; Chylinski et al., *RNA Biol*. 2013 May;10(5):726-37; Ma et al., *Biomed Res Int*. 2013;2013:270805; Hou et al., *Proc Natl Acad Sci U S A*. 2013 Sep 24;110(39):15644-9; Jinek et al., *Elife*. 2013;2:e00471; Pattanayak et al., *Nat Biotechnol*. 2013 Sep;31(9):839-43; Qi et al., *Cell*. 2013 Feb 28;152(5):1173-83; Wang et al., *Cell*. 2013 May 9;153(4):910-8; Auer et al., *Genome Res*. 2013 Oct 31; Chen et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e19; Cheng et al., *Cell Res*. 2013 Oct;23(10):1163-71; Cho et al., *Genetics*. 2013 Nov;195(3):1177-80; DiCarlo et al., *Nucleic Acids Res*. 2013 Apr;41(7):4336-43; Dickinson et al., *Nat Methods*. 2013 Oct;10(10):1028-34; Ebina et al., *Sci Rep*. 2013;3:2510; Fujii et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e187; Hu et al., *Cell Res*. 2013 Nov;23(11):1322-5; Jiang et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e188; Larson et al., *Nat Protoc*. 2013 Nov;8(11):2180-96; Mali et al.,

Nat Methods. 2013 Oct;10(10):957-63; Nakayama et al., Genesis. 2013 Dec;51(12):835-43; Ran et al., Nat Protoc. 2013 Nov;8(11):2281-308; Ran et al., Cell. 2013 Sep 12;154(6):1380-9; Upadhyay et al., G3 (Bethesda). 2013 Dec 9;3(12):2233-8; Walsh et al., Proc Natl Acad Sci U S A. 2013 Sep 24;110(39):15514-5; Xie et al., Mol Plant. 2013 Oct 9; Yang et al., Cell. 2013 Sep 12;154(6):1370-9; Briner et al., Mol Cell. 2014 Oct 23;56(2):333-9; and U.S. patents and patent applications: 8,906,616; 8,895,308; 8,889,418; 8,889,356; 8,871,445; 8,865,406; 8,795,965; 8,771,945; 8,697,359; 20140068797; 20140170753; 20140179006; 20140179770; 20140186843; 20140186919; 20140186958; 20140189896; 20140227787; 20140234972; 20140242664; 20140242699; 20140242700; 20140242702; 20140248702; 20140256046; 20140273037; 20140273226; 20140273230; 20140273231; 20140273232; 20140273233; 20140273234; 20140273235; 20140287938; 20140295556; 20140295557; 20140298547; 20140304853; 20140309487; 20140310828; 20140310830; 20140315985; 20140335063; 20140335620; 20140342456; 20140342457; 20140342458; 20140349400; 20140349405; 20140356867; 20140356956; 20140356958; 20140356959; 20140357523; 20140357530; 20140364333; and 20140377868; all of which are hereby incorporated by reference in their entirety.

Nucleic acid modifications

[00198] In some embodiments, a nucleic acid of the present disclosure (e.g., a guide RNA; e.g., a guide RNA present in a system of the present disclosure, a composition of the present disclosure, or a kit of the present disclosure) has one or more modifications, e.g., a base modification, a backbone modification, etc., to provide the nucleic acid with a new or enhanced feature (e.g., improved stability). A nucleoside is a base-sugar combination. The base portion of the nucleoside is normally a heterocyclic base. The two most common classes of such heterocyclic bases are the purines and the pyrimidines. Nucleotides are nucleosides that further include a phosphate group covalently linked to the sugar portion of the nucleoside. For those nucleosides that include a pentofuranosyl sugar, the phosphate group can be linked to the 2', the 3', or the 5' hydroxyl moiety of the sugar. In forming oligonucleotides, the phosphate groups covalently link adjacent nucleosides to one another to form a linear polymeric compound. In turn, the respective ends of this linear polymeric compound can be further joined to form a circular compound; however, linear compounds are suitable. In addition, linear compounds may have internal nucleotide base complementarity and may therefore fold in a manner as to produce a fully or partially double-stranded compound. Within oligonucleotides, the phosphate groups are commonly referred to as forming the internucleoside backbone of the oligonucleotide. The normal linkage or backbone of RNA and DNA is a 3' to 5' phosphodiester linkage.

[00199] Suitable nucleic acid modifications include, but are not limited to: 2' O-methyl modified nucleotides, 2' Fluoro modified nucleotides, locked nucleic acid (LNA) modified nucleotides,

peptide nucleic acid (PNA) modified nucleotides, nucleotides with phosphorothioate linkages, and a 5' cap (e.g., a 7-methylguanylate cap (m7G)). Additional details and additional modifications are described below.

[00200] A 2'-O-Methyl modified nucleotide (also referred to as 2'-O-Methyl RNA) is a naturally occurring modification of RNA found in tRNA and other small RNAs that arises as a post-transcriptional modification. Oligonucleotides can be directly synthesized that contain 2'-O-Methyl RNA. This modification increases T_m of RNA:RNA duplexes but results in only small changes in RNA:DNA stability. It is stable with respect to attack by single-stranded ribonucleases and is typically 5 to 10-fold less susceptible to DNases than DNA. It is commonly used in antisense oligos as a means to increase stability and binding affinity to the target message.

[00201] 2' Fluoro modified nucleotides (e.g., 2' Fluoro bases) have a fluorine modified ribose which increases binding affinity (T_m) and also confers some relative nuclease resistance when compared to native RNA. These modifications are commonly employed in ribozymes and siRNAs to improve stability in serum or other biological fluids.

[00202] LNA bases have a modification to the ribose backbone that locks the base in the C3'-endo position, which favors RNA A-type helix duplex geometry. This modification significantly increases T_m and is also very nuclease resistant. Multiple LNA insertions can be placed in an oligo at any position except the 3'-end. Applications have been described ranging from antisense oligos to hybridization probes to SNP detection and allele specific PCR. Due to the large increase in T_m conferred by LNAs, they also can cause an increase in primer dimer formation as well as self-hairpin formation. In some cases, the number of LNAs incorporated into a single oligo is 10 bases or less.

[00203] The phosphorothioate (PS) bond (i.e., a phosphorothioate linkage) substitutes a sulfur atom for a non-bridging oxygen in the phosphate backbone of a nucleic acid (e.g., an oligo). This modification renders the internucleotide linkage resistant to nuclease degradation. Phosphorothioate bonds can be introduced between the last 3-5 nucleotides at the 5'- or 3'-end of the oligo to inhibit exonuclease degradation. Including phosphorothioate bonds within the oligo (e.g., throughout the entire oligo) can help reduce attack by endonucleases as well.

[00204] In some embodiments, a guide RNA has one or more nucleotides that are 2'-O-Methyl modified nucleotides. In some embodiments, a guide RNA has one or more 2' Fluoro modified nucleotides. In some embodiments, a guide RNA has one or more LNA bases. In some embodiments, a guide RNA has one or more nucleotides that are linked by a phosphorothioate bond (i.e., a guide RNA has one or more phosphorothioate linkages). In some embodiments, a

guide RNA has a 5' cap (e.g., a 7-methylguanylate cap (m7G)). In some embodiments, a guide RNA has a combination of modified nucleotides. For example, a guide RNA can have a 5' cap (e.g., a 7-methylguanylate cap (m7G)) in addition to having one or more nucleotides with other modifications (e.g., a 2'-O-Methyl nucleotide and/or a 2' Fluoro modified nucleotide and/or a LNA base and/or a phosphorothioate linkage).

Modified backbones and modified internucleoside linkages

- [00205] Examples of a guide RNAs containing modifications include a guide RNAs containing modified backbones or non-natural internucleoside linkages. Guide RNAs having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone.
- [00206] Suitable modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, phosphorodiamidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage. Suitable oligonucleotides having inverted polarity comprise a single 3' to 3' linkage at the 3'-most internucleotide linkage i.e. a single inverted nucleoside residue which may be a basic (the nucleobase is missing or has a hydroxyl group in place thereof). Various salts (such as, for example, potassium or sodium), mixed salts and free acid forms are also included.
- [00207] In some embodiments, a subject nucleic acid (e.g., a guide RNA) comprises one or more phosphorothioate and/or heteroatom internucleoside linkages, in particular -CH₂-NH-O-CH₂-, -CH₂-N(CH₃)-O-CH₂- (known as a methylene (methylimino) or MMI backbone), -CH₂-O-N(CH₃)-CH₂-, -CH₂-N(CH₃)-N(CH₃)-CH₂- and -O-N(CH₃)-CH₂-CH₂- (wherein the native phosphodiester internucleotide linkage is represented as -O-P(=O)(OH)-O-CH₂-). MMI type internucleoside linkages are disclosed in the above referenced U.S. Pat. No. 5,489,677, the disclosure of which is incorporated herein by reference in its entirety. Suitable amide internucleoside linkages are disclosed in U.S. Pat. No. 5,602,240, the disclosure of which is incorporated herein by reference in its entirety.
- [00208] Also suitable are nucleic acids (e.g., a guide RNA) having morpholino backbone structures as described in, e.g., U.S. Pat. No. 5,034,506. For example, in some embodiments, a subject nucleic acid comprises a 6-membered morpholino ring in place of a ribose ring. In some

of these embodiments, a phosphorodiamidate or other non-phosphodiester internucleoside linkage replaces a phosphodiester linkage.

[00209] Suitable modified polynucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

Mimetics

[00210] A subject nucleic acid (e.g., a guide RNA) can be a nucleic acid mimetic. The term "mimetic" as it is applied to polynucleotides is intended to include polynucleotides wherein only the furanose ring or both the furanose ring and the internucleotide linkage are replaced with non-furanose groups, replacement of only the furanose ring is also referred to in the art as being a sugar surrogate. The heterocyclic base moiety or a modified heterocyclic base moiety is maintained for hybridization with an appropriate target nucleic acid. One such nucleic acid, a polynucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA, the sugar-backbone of a polynucleotide is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleotides are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone.

[00211] One polynucleotide mimetic that has been reported to have excellent hybridization properties is a peptide nucleic acid (PNA). The backbone in PNA compounds is two or more linked aminoethylglycine units which gives PNA an amide containing backbone. The heterocyclic base moieties are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that describe the preparation of PNA compounds include, but are not limited to: U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, the disclosures of which are incorporated herein by reference in their entirety.

[00212] Another class of polynucleotide mimetic that has been studied is based on linked morpholino units (morpholino nucleic acid) having heterocyclic bases attached to the morpholino ring. A number of linking groups have been reported that link the morpholino monomeric units in a morpholino nucleic acid. One class of linking groups has been selected to

give a non-ionic oligomeric compound. The non-ionic morpholino-based oligomeric compounds are less likely to have undesired interactions with cellular proteins. Morpholino-based polynucleotides are non-ionic mimics of oligonucleotides which are less likely to form undesired interactions with cellular proteins (Dwayne A. Braasch and David R. Corey, *Biochemistry*, 2002, 41(14), 4503-4510). Morpholino-based polynucleotides are disclosed in U.S. Pat. No. 5,034,506, the disclosure of which is incorporated herein by reference in its entirety. A variety of compounds within the morpholino class of polynucleotides have been prepared, having a variety of different linking groups joining the monomeric subunits.

[00213] A further class of polynucleotide mimetic is referred to as cyclohexenyl nucleic acids (CeNA). The furanose ring normally present in a DNA/RNA molecule is replaced with a cyclohexenyl ring. CeNA DMT protected phosphoramidite monomers have been prepared and used for oligomeric compound synthesis following classical phosphoramidite chemistry. Fully modified CeNA oligomeric compounds and oligonucleotides having specific positions modified with CeNA have been prepared and studied (see Wang et al., *J. Am. Chem. Soc.*, 2000, 122, 8595-8602, the disclosure of which is incorporated herein by reference in its entirety). In general the incorporation of CeNA monomers into a DNA chain increases its stability of a DNA/RNA hybrid. CeNA oligoadenylates formed complexes with RNA and DNA complements with similar stability to the native complexes. The study of incorporating CeNA structures into natural nucleic acid structures was shown by NMR and circular dichroism to proceed with easy conformational adaptation.

[00214] A further modification includes Locked Nucleic Acids (LNAs) in which the 2'-hydroxyl group is linked to the 4' carbon atom of the sugar ring thereby forming a 2'-C,4'-C-oxymethylene linkage thereby forming a bicyclic sugar moiety. The linkage can be a methylene (-CH₂-), group bridging the 2' oxygen atom and the 4' carbon atom wherein n is 1 or 2 (Singh et al., *Chem. Commun.*, 1998, 4, 455-456, the disclosure of which is incorporated herein by reference in its entirety). LNA and LNA analogs display very high duplex thermal stabilities with complementary DNA and RNA (T_m=+3 to +10° C), stability towards 3'-exonucleolytic degradation and good solubility properties. Potent and nontoxic antisense oligonucleotides containing LNAs have been described (e.g., Wahlestedt et al., *Proc. Natl. Acad. Sci. U.S.A.*, 2000, 97, 5633-5638, the disclosure of which is incorporated herein by reference in its entirety).

[00215] The synthesis and preparation of the LNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (e.g., Koshkin et al., *Tetrahedron*, 1998, 54, 3607-3630, the disclosure of which is incorporated herein by reference in its entirety). LNAs and preparation thereof are also described in WO 98/39352 and WO 99/14226, as well as U.S.

applications 20120165514, 20100216983, 20090041809, 20060117410, 20040014959, 20020094555, and 20020086998, the disclosures of which are incorporated herein by reference in their entirety.

Modified sugar moieties

[00216] A subject nucleic acid (e.g., a guide RNA) can also include one or more substituted sugar moieties. Suitable polynucleotides comprise a sugar substituent group selected from: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀ alkyl or C₂ to C₁₀ alkenyl and alkynyl. Particularly suitable are O((CH₂)_nO)_mCH₃, O(CH₂)_nOCH₃, O(CH₂)_nNH₂, O(CH₂)_nCH₃, O(CH₂)_nONH₂, and O(CH₂)_nON((CH₂)_nCH₃)₂, where n and m are from 1 to about 10. Other suitable polynucleotides comprise a sugar substituent group selected from: C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkenyl, alkynyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH₃, OCN, Cl, Br, CN, CF₃, OCF₃, SOCH₃, SO₂CH₃, ONO₂, NO₂, N₃, NH₂, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A suitable modification includes 2'-methoxyethoxy (2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486-504, the disclosure of which is incorporated herein by reference in its entirety) i.e., an alkoxyalkoxy group. A further suitable modification includes 2'-dimethylaminoethoxyethoxy, i.e., a O(CH₂)₂ON(CH₃)₂ group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethyl-amino-ethoxy-ethyl or 2'-DMAEOE), i.e., 2'-O-CH₂-O-CH₂-N(CH₃)₂.

[00217] Other suitable sugar substituent groups include methoxy (-O-CH₃), aminopropoxy (--O-CH₂CH₂CH₂NH₂), allyl (-CH₂-CH=CH₂), -O-allyl (--O-CH₂-CH=CH₂) and fluoro (F). 2'-sugar substituent groups may be in the arabino (up) position or ribo (down) position. A suitable 2'-arabino modification is 2'-F. Similar modifications may also be made at other positions on the oligomeric compound, particularly the 3' position of the sugar on the 3' terminal nucleoside or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Oligomeric compounds may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

Base modifications and substitutions

[00218] A subject nucleic acid (e.g., a guide RNA) may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine

bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl ($-\text{C}\equiv\text{C}-\text{CH}_3$) uracil and cytosine and other alkynyl derivatives of pyrimidine bases, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 2-F-adenine, 2-amino-adenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further modified nucleobases include tricyclic pyrimidines such as phenoxazine cytidine (1H-pyrimido(5,4-b)(1,4)benzoxazin-2(3H)-one), phenothiazine cytidine (1H-pyrimido(5,4-b)(1,4)benzothiazin-2(3H)-one), G-clamps such as a substituted phenoxazine cytidine (e.g. 9-(2-aminoethoxy)-H-pyrimido(5,4-b)(1,4)benzoxazin-2(3H)-one), carbazole cytidine (2H-pyrimido(4,5-b)indol-2-one), pyridoindole cytidine (H-pyrido(3',2':4,5)pyrrolo(2,3-d)pyrimidin-2-one).

[00219] Heterocyclic base moieties may also include those in which the purine or pyrimidine base is replaced with other heterocycles, for example 7-deaza-adenine, 7-deazaguanosine, 2-aminopyridine and 2-pyridone. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in *The Concise Encyclopedia Of Polymer Science And Engineering*, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., *Chapter 15, Antisense Research and Applications*, pages 289-302, Crooke, S. T. and Lebleu, B., ed., CRC Press, 1993; the disclosures of which are incorporated herein by reference in their entirety. Certain of these nucleobases are useful for increasing the binding affinity of an oligomeric compound. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi et al., eds., *Antisense Research and Applications*, CRC Press, Boca Raton, 1993, pp. 276-278; the disclosure of which is incorporated herein by reference in its entirety) and are suitable base substitutions, e.g., when combined with 2'-O-methoxyethyl sugar modifications.

Conjugates

[00220] Another possible modification of a subject nucleic acid involves chemically linking to the polynucleotide one or more moieties or conjugates which enhance the activity, cellular

distribution or cellular uptake of the oligonucleotide. These moieties or conjugates can include conjugate groups covalently bound to functional groups such as primary or secondary hydroxyl groups. Conjugate groups include, but are not limited to, intercalators, reporter molecules, polyamines, polyamides, polyethylene glycols, polyethers, groups that enhance the pharmacodynamic properties of oligomers, and groups that enhance the pharmacokinetic properties of oligomers. Suitable conjugate groups include, but are not limited to, cholesterol, lipids, phospholipids, biotin, phenazine, folate, phenanthridine, anthraquinone, acridine, fluoresceins, rhodamines, coumarins, and dyes. Groups that enhance the pharmacodynamic properties include groups that improve uptake, enhance resistance to degradation, and/or strengthen sequence-specific hybridization with the target nucleic acid. Groups that enhance the pharmacokinetic properties include groups that improve uptake, distribution, metabolism or excretion of a subject nucleic acid.

[00221] Conjugate moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553-6556), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306-309; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 1111-1118; Kabanov et al., *FEBS Lett.*, 1990, 259, 327-330; Svinarchuk et al., *Biochimie*, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923-937).

[00222] A conjugate may include a "Protein Transduction Domain" or PTD (also known as a CPP – cell penetrating peptide), which may refer to a polypeptide, polynucleotide, carbohydrate, or organic or inorganic compound that facilitates traversing a lipid bilayer, micelle, cell membrane, organelle membrane, or vesicle membrane. A PTD attached to another molecule, which can range from a small polar molecule to a large macromolecule and/or a nanoparticle, facilitates the molecule traversing a membrane, for example going from extracellular space to intracellular space, or cytosol to within an organelle (e.g., the nucleus). In some embodiments, a PTD is covalently linked to the 3' end of an exogenous polynucleotide. In some embodiments, a

PTD is covalently linked to the 5' end of an exogenous polynucleotide. Exemplary PTDs include but are not limited to a minimal undecapeptide protein transduction domain (corresponding to residues 47-57 of HIV-1 TAT comprising YGRKKRRQRRR; SEQ ID NO:854); a polyarginine sequence comprising a number of arginines sufficient to direct entry into a cell (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines); a VP22 domain (Zender et al. (2002) *Cancer Gene Ther.* 9(6):489-96); an *Drosophila* Antennapedia protein transduction domain (Noguchi et al. (2003) *Diabetes* 52(7):1732-1737); a truncated human calcitonin peptide (Trehin et al. (2004) *Pharm. Research* 21:1248-1256); polylysine (Wender et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:13003-13008); RRQRRTSKLMKR SEQ ID NO:855); Transportan GWTLNSAGYLLGKINLKALAALAKKIL SEQ ID NO:856); KALAWKAKLAKALAKALAKHLAKALAKALKCEA SEQ ID NO:857); and RQIKIWFQNRRMKWKK SEQ ID NO:853). Exemplary PTDs include but are not limited to, YGRKKRRQRRR SEQ ID NO:854), RKKRRQRRR SEQ ID NO:858); an arginine homopolymer of from 3 arginine residues to 50 arginine residues; Exemplary PTD domain amino acid sequences include, but are not limited to, any of the following: YGRKKRRQRRR SEQ ID NO:854); RKKRRQRRR SEQ ID NO:859); YARAAARQARA SEQ ID NO:860); THRLPRRRRRR SEQ ID NO:861); and GGRRARRRRRRR SEQ ID NO:862). In some embodiments, the PTD is an activatable CPP (ACPP) (Aguilera et al. (2009) *Integr Biol (Camb)* June; 1(5-6): 371-381). ACPPs comprise a polycationic CPP (e.g., Arg9 or "R9") connected via a cleavable linker to a matching polyanion (e.g., Glu9 or "E9"), which reduces the net charge to nearly zero and thereby inhibits adhesion and uptake into cells. Upon cleavage of the linker, the polyanion is released, locally unmasking the polyarginine and its inherent adhesiveness, thus "activating" the ACPP to traverse the membrane.

Introducing components into a target cell

[00223] A guide RNA (or a nucleic acid comprising a nucleotide sequence encoding same) and/or an RNA-guided effector polypeptide of the present disclosure (or a nucleic acid comprising a nucleotide sequence encoding same) and/or a fusion polypeptide of the present disclosure (or a nucleic acid that includes a nucleotide sequence encoding a fusion polypeptide of the present disclosure) and/or a donor polynucleotide (donor template) can be introduced into a host cell by any of a variety of well-known methods.

[00224] Any of a variety of compounds and methods can be used to deliver to a target cell a system of the present disclosure (e.g., where a system of the present disclosure comprises: a) an RNA-guided effector polypeptide of the present disclosure and a guide RNA; b) a fusion polypeptide of the present disclosure and a guide RNA; c) a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; d) an mRNA encoding an RNA-guided effector polypeptide of the present disclosure; and a guide RNA; e) an mRNA encoding a

fusion polypeptide of the present disclosure; and a guide RNA; f) an mRNA encoding a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; g) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; h) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; i) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a guide RNA, and a nucleotide sequence encoding a donor template nucleic acid; j) a first recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; k) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; l) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; and a donor template nucleic acid; m) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or n) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or some variation of one of (a) through (n). As a non-limiting example, a system of the present disclosure can be combined with a lipid. As another non-limiting example, a system of the present disclosure can be combined with a particle, or formulated into a particle.

[00225] Methods of introducing a nucleic acid into a host cell are known in the art, and any convenient method can be used to introduce a subject nucleic acid (e.g., an expression construct/vector) into a target cell (e.g., prokaryotic cell, eukaryotic cell, plant cell, animal cell, mammalian cell, human cell, and the like). Suitable methods include, e.g., viral infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro injection, nanoparticle-mediated nucleic acid delivery (see, e.g., Panyam et., al *Adv Drug Deliv Rev.* 2012 Sep 13. pii: S0169-409X(12)00283-9. doi: 10.1016/j.addr.2012.09.023), and the like.

[00226] In some cases, an RNA-guided effector polypeptide of the present disclosure is provided as a nucleic acid (e.g., an mRNA, a DNA, a plasmid, an expression vector, a viral vector, etc.) that encodes the RNA-guided effector polypeptide. In some cases, the RNA-guided effector polypeptide of the present disclosure is provided directly as a protein (e.g., without an associated guide RNA or with an associate guide RNA, i.e., as a ribonucleoprotein complex). An RNA-guided effector polypeptide of the present disclosure can be introduced into a cell (provided to the cell) by any convenient method; such methods are known to those of ordinary skill in the art. As an illustrative example, an RNA-guided effector polypeptide of the present disclosure can be injected directly into a cell (e.g., with or without a guide RNA or nucleic acid encoding a guide RNA, and with or without a donor polynucleotide). As another example, a preformed complex of an RNA-guided effector polypeptide of the present disclosure and a guide RNA (an RNP) can be introduced into a cell (e.g., eukaryotic cell) (e.g., via injection, via nucleofection; via a protein transduction domain (PTD) conjugated to one or more components, e.g., conjugated to the RNA-guided effector polypeptide protein, conjugated to a guide RNA, conjugated to an RNA-guided effector polypeptide of the present disclosure and a guide RNA; etc.).

[00227] In some cases, a fusion polypeptide (an RNA-guided effector polypeptide fused to a heterologous fusion partner) of the present disclosure is provided as a nucleic acid (e.g., an mRNA, a DNA, a plasmid, an expression vector, a viral vector, etc.) that encodes the fusion polypeptide. In some cases, a fusion polypeptide of the present disclosure is provided directly as a protein (e.g., without an associated guide RNA or with an associate guide RNA, i.e., as a ribonucleoprotein complex). A fusion polypeptide of the present disclosure can be introduced into a cell (provided to the cell) by any convenient method; such methods are known to those of ordinary skill in the art. As an illustrative example, a fusion polypeptide of the present disclosure can be injected directly into a cell (e.g., with or without nucleic acid encoding a guide RNA and with or without a donor polynucleotide). As another example, a preformed complex of a fusion polypeptide of the present disclosure and a guide RNA (an RNP) can be introduced into a cell (e.g., via injection, via nucleofection; via a protein transduction domain (PTD) conjugated to one or more components, e.g., conjugated to the fusion protein, conjugated to a guide RNA, conjugated to a fusion polypeptide of the present disclosure and a guide RNA; etc.).

[00228] In some cases, a nucleic acid (e.g., a guide RNA; a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure; etc.) is delivered to a cell (e.g., a target host cell) and/or a polypeptide (e.g., an RNA-guided effector polypeptide; a fusion polypeptide) in a particle, or associated with a particle. In some cases, a system of the present disclosure is delivered to a cell in a particle, or associated with a particle.

The terms “particle” and “nanoparticle” can be used interchangeable, as appropriate. A recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure and/or a guide RNA, an mRNA comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure or a fusion polypeptide of the present disclosure, and guide RNA may be delivered simultaneously using particles or lipid envelopes; for instance, an RNA-guided effector polypeptide of the present disclosure polypeptide and a guide RNA, or a fusion polypeptide of the present disclosure and a guide RNA, e.g., as a complex (e.g., a ribonucleoprotein (RNP) complex), can be delivered via a particle, e.g., a delivery particle comprising lipid or lipidoid and hydrophilic polymer, e.g., a cationic lipid and a hydrophilic polymer, for instance wherein the cationic lipid comprises 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) or 1,2-ditetradecanoyl-sn-glycero-3-phosphocholine (DMPC) and/or wherein the hydrophilic polymer comprises ethylene glycol or polyethylene glycol (PEG); and/or wherein the particle further comprises cholesterol (e.g., particle from formulation 1=DOTAP 100, DMPC 0, PEG 0, Cholesterol 0; formulation number 2=DOTAP 90, DMPC 0, PEG 10, Cholesterol 0; formulation number 3=DOTAP 90, DMPC 0, PEG 5, Cholesterol 5). For example, a particle can be formed using a multistep process in which an RNA-guided effector polypeptide of the present disclosure and a guide RNA, or a fusion polypeptide of the present disclosure and a guide RNA, are mixed together, e.g., at a 1:1 molar ratio, e.g., at room temperature, e.g., for 30 minutes, e.g., in sterile, nuclease free 1 x phosphate-buffered saline (PBS); and separately, DOTAP, DMPC, PEG, and cholesterol as applicable for the formulation are dissolved in alcohol, e.g., 100% ethanol; and, the two solutions are mixed together to form particles containing the complexes).

[00229] A RNA-guided effector polypeptide of the present disclosure polypeptide (or an mRNA comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure polypeptide; or a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure polypeptide) and/or guide RNA (or a nucleic acid such as one or more expression vectors encoding the guide RNA), or a fusion polypeptide of the present disclosure (or an mRNA comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure; or a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure polypeptide) and/or a guide RNA may be delivered simultaneously using particles or lipid envelopes. For example, a biodegradable core-shell structured nanoparticle with a poly (β -amino ester) (PBAE) core enveloped by a phospholipid bilayer shell can be used. In some cases, particles/nanoparticles based on self assembling bioadhesive polymers are used; such

particles/nanoparticles may be applied to oral delivery of peptides, intravenous delivery of peptides and nasal delivery of peptides, e.g., to the brain. Other embodiments, such as oral absorption and ocular delivery of hydrophobic drugs are also contemplated. A molecular envelope technology, which involves an engineered polymer envelope which is protected and delivered to the site of the disease, can be used. Doses of about 5 mg/kg can be used, with single or multiple doses, depending on various factors, e.g., the target tissue.

[00230] Lipidoid compounds (e.g., as described in US patent application 20110293703) are also useful in the administration of polynucleotides, and can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure (e.g., where a system of the present disclosure comprises: a) an RNA-guided effector polypeptide of the present disclosure and a guide RNA; b) a fusion polypeptide of the present disclosure and a guide RNA; c) a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; d) an mRNA encoding an RNA-guided effector polypeptide of the present disclosure; and a guide RNA; e) an mRNA encoding a fusion polypeptide of the present disclosure; and a guide RNA; f) an mRNA encoding a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; g) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; h) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; i) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a guide RNA, and a nucleotide sequence encoding a donor template nucleic acid; j) a first recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; k) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; l) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; and a donor template nucleic acid; m) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or n) a recombinant expression vector

comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or some variation of one of (a) through (n). In one aspect, the aminoalcohol lipidoid compounds are combined with an agent to be delivered to a cell or a subject to form microparticles, nanoparticles, liposomes, or micelles. The aminoalcohol lipidoid compounds may be combined with other aminoalcohol lipidoid compounds, polymers (synthetic or natural), surfactants, cholesterol, carbohydrates, proteins, lipids, etc. to form the particles. These particles may then optionally be combined with a pharmaceutical excipient to form a pharmaceutical composition.

[00231] A poly(beta-amino alcohol) (PBAA) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. US Patent Publication No. 20130302401 relates to a class of poly(beta-amino alcohols) (PBAA) that has been prepared using combinatorial polymerization.

[00232] Sugar-based particles may be used, for example GalNAc, as described with reference to WO2014118272 (incorporated herein by reference) and Nair, J K et al., 2014, Journal of the American Chemical Society 136 (49), 16958-16961) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell.

[00233] In some cases, lipid nanoparticles (LNPs) are used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. Negatively charged polymers such as RNA may be loaded into LNPs at low pH values (e.g., pH 4) where the ionizable lipids display a positive charge. However, at physiological pH values, the LNPs exhibit a low surface charge compatible with longer circulation times. Four species of ionizable cationic lipids have been focused upon, namely 1,2-dilinoylel-3-dimethylammonium-propane (DLinDAP), 1,2-dilinoyleloxy-3-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinoyleloxy-keto-N,N-dimethyl-3-aminopropane (DLinKDMA), and 1,2-dilinoylel-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLinKC2-DMA). Preparation of LNPs and is described in, e.g., Rosin et al. (2011) Molecular Therapy 19:1286-2200). The cationic lipids 1,2-dilinoylel-3-dimethylammonium-propane (DLinDAP), 1,2-dilinoyleloxy-3-N,N-dimethylaminopropane (DLinDMA), 1,2-dilinoyleloxyketo-N,N-dimethyl-3-aminopropane (DLinK-DMA), 1,2-dilinoylel-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLinKC2-DMA), (3-o-[2"-(methoxypolyethyleneglycol 2000) succinoyl]-1,2-dimyristoyl-sn-

glycol (PEG-S-DMG), and R-3-[(omega-methoxy-poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxypropyl-3-amine (PEG-C-DOMG) may be used. A nucleic acid (e.g., a guide RNA; a nucleic acid of the present disclosure; etc.) may be encapsulated in LNPs containing DLinDAP, DLinDMA, DLinK-DMA, and DLinKC2-DMA (cationic lipid:DSPC:CHOL: PEGS-DMG or PEG-C-DOMG at 40:10:40:10 molar ratios). In some cases, 0.2% SP-DiOC18 is incorporated.

[00234] Spherical Nucleic Acid (SNATM) constructs and other nanoparticles (particularly gold nanoparticles) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell.. See, e.g., Cutler et al., J. Am. Chem. Soc. 2011 133:9254-9257, Hao et al., Small. 2011 7:3158-3162, Zhang et al., ACS Nano. 2011 5:6962-6970, Cutler et al., J. Am. Chem. Soc. 2012 134:1376-1391, Young et al., Nano Lett. 2012 12:3867-71, Zheng et al., Proc. Natl. Acad. Sci. USA. 2012 109:11975-80, Mirkin, Nanomedicine 2012 7:635-638 Zhang et al., J. Am. Chem. Soc. 2012 134:16488-1691, Weintraub, Nature 2013 495:S14-S16, Choi et al., Proc. Natl. Acad. Sci. USA. 2013 110(19): 7625-7630, Jensen et al., Sci. Transl. Med. 5, 209ra152 (2013) and Mirkin, et al., Small, 10:186-192.

[00235] Self-assembling nanoparticles with RNA may be constructed with polyethyleneimine (PEI) that is PEGylated with an Arg-Gly-Asp (RGD) peptide ligand attached at the distal end of the polyethylene glycol (PEG).

[00236] In general, a "nanoparticle" refers to any particle having a diameter of less than 1000 nm. In some cases, nanoparticles suitable for use in delivering an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell have a diameter of 500 nm or less, e.g., from 25 nm to 35 nm, from 35 nm to 50 nm, from 50 nm to 75 nm, from 75 nm to 100 nm, from 100 nm to 150 nm, from 150 nm to 200 nm, from 200 nm to 300 nm, from 300 nm to 400 nm, or from 400 nm to 500 nm. In some cases, nanoparticles suitable for use in delivering an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell have a diameter of from 25 nm to 200 nm. In some cases, nanoparticles suitable for use in delivering an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell have a diameter of 100 nm or less In some cases, nanoparticles suitable for use in delivering an RNA-guided effector

polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell have a diameter of from 35 nm to 60 nm.

[00237] Nanoparticles suitable for use in delivering an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell may be provided in different forms, e.g., as solid nanoparticles (e.g., metal such as silver, gold, iron, titanium), non-metal, lipid-based solids, polymers), suspensions of nanoparticles, or combinations thereof. Metal, dielectric, and semiconductor nanoparticles may be prepared, as well as hybrid structures (e.g., core-shell nanoparticles). Nanoparticles made of semiconducting material may also be labeled quantum dots if they are small enough (typically below 10 nm) that quantization of electronic energy levels occurs. Such nanoscale particles are used in biomedical applications as drug carriers or imaging agents and may be adapted for similar purposes in the present disclosure.

[00238] Semi-solid and soft nanoparticles are also suitable for use in delivering polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. A prototype nanoparticle of semi-solid nature is the liposome.

[00239] In some cases, an exosome is used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. Exosomes are endogenous nano-vesicles that transport RNAs and proteins, and which can deliver RNA to the brain and other target organs.

[00240] In some cases, a liposome is used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. Liposomes are spherical vesicle structures composed of a uni- or multilamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer. Liposomes can be made from several different types of lipids; however, phospholipids are most commonly used to generate liposomes. Although liposome formation is spontaneous when a lipid film is mixed with an aqueous solution, it can also be expedited by applying force in the form of shaking by using a homogenizer, sonicator, or an extrusion apparatus. Several other additives may be added to liposomes in order to modify their structure and properties. For instance, either cholesterol or sphingomyelin may be added to the liposomal mixture in order to help stabilize the liposomal structure and to prevent the leakage of the

liposomal inner cargo. A liposome formulation may be mainly comprised of natural phospholipids and lipids such as 1,2-distearoyl-sn-glycero-3-phosphatidyl choline (DSPC), sphingomyelin, egg phosphatidylcholines and monosialoganglioside.

[00241] A stable nucleic-acid-lipid particle (SNALP) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. The SNALP formulation may contain the lipids 3-N-[(methoxypoly(ethylene glycol) 2000) carbamoyl]-1,2-dimyristyloxy-propylamine (PEG-C-DMA), 1,2-dilinoleyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and cholesterol, in a 2:40:10:48 molar percent ratio. The SNALP liposomes may be prepared by formulating D-Lin-DMA and PEG-C-DMA with distearoylphosphatidylcholine (DSPC), Cholesterol and siRNA using a 25:1 lipid/siRNA ratio and a 48/40/10/2 molar ratio of Cholesterol/D-Lin-DMA/DSPC/PEG-C-DMA. The resulting SNALP liposomes can be about 80-100 nm in size. A SNALP may comprise synthetic cholesterol (Sigma-Aldrich, St Louis, Mo., USA), dipalmitoylphosphatidylcholine (Avanti Polar Lipids, Alabaster, Ala., USA), 3-N-[(w-methoxy poly(ethylene glycol)2000)carbamoyl]-1,2-dimyrestyloxypropylamine, and cationic 1,2-dilinoleyloxy-3-N,Ndimethylaminopropane. A SNALP may comprise synthetic cholesterol (Sigma-Aldrich), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC; Avanti Polar Lipids Inc.), PEG-cDMA, and 1,2-dilinoleyloxy-3-(N;N-dimethyl)aminopropane (DLinDMA).

[00242] Other cationic lipids, such as amino lipid 2,2-dilinoley-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. A preformed vesicle with the following lipid composition may be contemplated: amino lipid, distearoylphosphatidylcholine (DSPC), cholesterol and (R)-2,3-bis(octadecyloxy)propyl-1-(methoxy poly(ethylene glycol)2000)propylcarbamate (PEG-lipid) in the molar ratio 40/10/40/10, respectively, and a FVII siRNA/total lipid ratio of approximately 0.05 (w/w). To ensure a narrow particle size distribution in the range of 70-90 nm and a low polydispersity index of 0.11.+-.0.04 (n=56), the particles may be extruded up to three times through 80 nm membranes prior to adding the guide RNA. Particles containing the highly potent amino lipid 16 may be used, in which the molar ratio of the four lipid components 16, DSPC, cholesterol and PEG-lipid (50/10/38.5/1.5) which may be further optimized to enhance in vivo activity.

[00243] Lipids may be formulated with a system of the present disclosure or component(s) thereof or nucleic acids encoding the same to form lipid nanoparticles (LNPs). Suitable lipids

include, but are not limited to, DLin-KC2-DMA4, C12-200 and colipids distearylphosphatidyl choline, cholesterol, and PEG-DMG may be formulated with a system, or component thereof, of the present disclosure, using a spontaneous vesicle formation procedure. The component molar ratio may be about 50/10/38.5/1.5 (DLin-KC2-DMA or C12-200/distearylphosphatidyl choline/cholesterol/PEG-DMG).

- [00244]** A system of the present disclosure, or a component thereof, may be delivered encapsulated in PLGA microspheres such as that further described in US published applications 20130252281 and 20130245107 and 20130244279.
- [00245]** Supercharged proteins can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. Supercharged proteins are a class of engineered or naturally occurring proteins with unusually high positive or negative net theoretical charge. Both supernegatively and superpositively charged proteins exhibit the ability to withstand thermally or chemically induced aggregation. Superpositively charged proteins are also able to penetrate mammalian cells. Associating cargo with these proteins, such as plasmid DNA, RNA, or other proteins, can facilitate the functional delivery of these macromolecules into mammalian cells both *in vitro* and *in vivo*.
- [00246]** Cell Penetrating Peptides (CPPs) can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell. CPPs typically have an amino acid composition that either contains a high relative abundance of positively charged amino acids such as lysine or arginine or has sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids.
- [00247]** An implantable device can be used to deliver an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure (e.g., a guide RNA, a nucleic acid encoding a guide RNA, a nucleic acid encoding an RNA-guided effector polypeptide, a donor template, and the like), or a system of the present disclosure, to a target cell (e.g., a target cell *in vivo*, where the target cell is a target cell in circulation, a target cell in a tissue, a target cell in an organ, etc.). An implantable device suitable for use in delivering an RNA-guided effector polypeptide of the present disclosure, a fusion polypeptide of the present disclosure, an RNP of the present disclosure, a nucleic acid of the present disclosure, or a system of the present disclosure, to a target cell (e.g., a target cell *in vivo*, where the target cell is a target cell in circulation, a target

cell in a tissue, a target cell in an organ, etc.) can include a container (e.g., a reservoir, a matrix, etc.) that comprises the RNA-guided effector polypeptide, the fusion polypeptide, the RNP, or the system (or component thereof, e.g., a nucleic acid of the present disclosure).

[00248] A suitable implantable device can comprise a polymeric substrate, such as a matrix for example, that is used as the device body, and in some cases additional scaffolding materials, such as metals or additional polymers, and materials to enhance visibility and imaging. An implantable delivery device can be advantageous in providing release locally and over a prolonged period, where the polypeptide and/or nucleic acid to be delivered is released directly to a target site, e.g., the extracellular matrix (ECM), the vasculature surrounding a tumor, a diseased tissue, etc. Suitable implantable delivery devices include devices suitable for use in delivering to a cavity such as the abdominal cavity and/or any other type of administration in which the drug delivery system is not anchored or attached, comprising a biostable and/or degradable and/or bioabsorbable polymeric substrate, which may for example optionally be a matrix. In some cases, a suitable implantable drug delivery device comprises degradable polymers, wherein the main release mechanism is bulk erosion. In some cases, a suitable implantable drug delivery device comprises non degradable, or slowly degraded polymers, wherein the main release mechanism is diffusion rather than bulk erosion, so that the outer part functions as membrane, and its internal part functions as a drug reservoir, which practically is not affected by the surroundings for an extended period (for example from about a week to about a few months). Combinations of different polymers with different release mechanisms may also optionally be used. The concentration gradient at the can be maintained effectively constant during a significant period of the total releasing period, and therefore the diffusion rate is effectively constant (termed "zero mode" diffusion). By the term "constant" it is meant a diffusion rate that is maintained above the lower threshold of therapeutic effectiveness, but which may still optionally feature an initial burst and/or may fluctuate, for example increasing and decreasing to a certain degree. The diffusion rate can be so maintained for a prolonged period, and it can be considered constant to a certain level to optimize the therapeutically effective period, for example the effective silencing period.

[00249] In some cases, the implantable delivery system is designed to shield the nucleotide based therapeutic agent from degradation, whether chemical in nature or due to attack from enzymes and other factors in the body of the subject.

[00250] The site for implantation of the device, or target site, can be selected for maximum therapeutic efficacy. For example, a delivery device can be implanted within or in the proximity of a tumor environment, or the blood supply associated with a tumor. The target location can be, e.g.: 1) the brain at degenerative sites like in Parkinson or Alzheimer disease at the basal ganglia,

white and gray matter; 2) the spine, as in the case of amyotrophic lateral sclerosis (ALS); 3) uterine cervix; 4) active and chronic inflammatory joints; 5) dermis as in the case of psoriasis; 6) sympathetic and sensoric nervous sites for analgesic effect; 7) a bone; 8) a site of acute or chronic infection; 9) Intra vaginal; 10) Inner ear--auditory system, labyrinth of the inner ear, vestibular system; 11) Intra tracheal; 12) Intra-cardiac; coronary, epicardiac; 13) urinary tract or bladder; 14) biliary system; 15) parenchymal tissue including and not limited to the kidney, liver, spleen; 16) lymph nodes; 17) salivary glands; 18) dental gums; 19) Intra-articular (into joints); 20) Intra-ocular; 21) Brain tissue; 22) Brain ventricles; 23) Cavities, including abdominal cavity (for example but without limitation, for ovary cancer); 24) Intra esophageal; and 25) Intra rectal; and 26) into the vasculature.

[00251] The method of insertion, such as implantation, may optionally already be used for other types of tissue implantation and/or for insertions and/or for sampling tissues, optionally without modifications, or alternatively optionally only with non-major modifications in such methods. Such methods optionally include but are not limited to brachytherapy methods, biopsy, endoscopy with and/or without ultrasound, such as stereotactic methods into the brain tissue, laparoscopy, including implantation with a laparoscope into joints, abdominal organs, the bladder wall and body cavities.

MODIFIED HOST CELLS

[00252] The present disclosure provides a modified cell comprising an RNA-guided effector polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a modified cell comprising an RNA-guided effector polypeptide of the present disclosure, where the modified cell is a cell that does not normally comprise the RNA-guided effector polypeptide. The present disclosure provides a modified cell (e.g., a genetically modified cell) comprising nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with an mRNA comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression vector comprising: a) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; and b) a nucleotide sequence encoding a guide RNA of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression

vector comprising: a) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; b) a nucleotide sequence encoding a guide RNA of the present disclosure; and c) a nucleotide sequence encoding a donor template.

[00253] The present disclosure provides a modified cell comprising a fusion polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure. The present disclosure provides a modified cell comprising a fusion polypeptide of the present disclosure, where the modified cell is a cell that does not normally comprise the fusion polypeptide. The present disclosure provides a modified cell (e.g., a genetically modified cell) comprising nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with an mRNA comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression vector comprising: a) a nucleotide sequence encoding a fusion polypeptide of the present disclosure; and b) a nucleotide sequence encoding a guide RNA of the present disclosure. The present disclosure provides a genetically modified cell that is genetically modified with a recombinant expression vector comprising: a) a nucleotide sequence encoding a fusion polypeptide of the present disclosure; b) a nucleotide sequence encoding a guide RNA of the present disclosure; and c) a nucleotide sequence encoding a donor template.

[00254] A cell that serves as a recipient for an RNA-guided effector polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and/or a guide RNA of the present disclosure, or that serves as a recipient for a fusion polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and/or a guide RNA of the present disclosure, can be any of a variety of cells, including, e.g., *in vitro* cells; *in vivo* cells; *ex vivo* cells; primary cells; cancer cells; animal cells; plant cells; algal cells; fungal cells; etc. A cell that serves as a recipient for an RNA-guided effector polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and/or a guide RNA of the present disclosure, or that serves as a recipient for a fusion polypeptide of the present disclosure and/or a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and/or a guide RNA of the present disclosure, is referred to as a “host cell” or a “target cell.” A host cell or a target cell can be a recipient of a system of the present disclosure.

A host cell or a target cell can be a recipient of an RNP of the present disclosure. A host cell or a target cell can be a recipient of a single component of a system of the present disclosure.

[00255] Non-limiting examples of cells (target cells) include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant (e.g., cells from plant crops, fruits, vegetables, grains, soy bean, corn, maize, wheat, seeds, tomatoes, rice, cassava, sugarcane, pumpkin, hay, potatoes, cotton, cannabis, tobacco, flowering plants, conifers, gymnosperms, angiosperms, ferns, clubmosses, hornworts, liverworts, mosses, dicotyledons, monocotyledons, etc.), an algal cell, (e.g., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens*, *C. agardh*, and the like), seaweeds (e.g. kelp) a fungal cell (e.g., a yeast cell, a cell from a mushroom), an animal cell, a cell from an invertebrate animal (e.g., fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., an ungulate (e.g., a pig, a cow, a goat, a sheep); a rodent (e.g., a rat, a mouse); a non-human primate; a human; a feline (e.g., a cat); a canine (e.g., a dog); etc.), and the like. In some cases, the cell is a cell that does not originate from a natural organism (e.g., the cell can be a synthetically made cell; also referred to as an artificial cell).

[00256] A cell can be an *in vitro* cell (e.g., established cultured cell line). A cell can be an *ex vivo* cell (cultured cell from an individual). A cell can be an *in vivo* cell (e.g., a cell in an individual). A cell can be an isolated cell. A cell can be a cell inside of an organism. A cell can be an organism. A cell can be a cell in a cell culture (e.g., *in vitro* cell culture). A cell can be one of a collection of cells. A cell can be a prokaryotic cell or derived from a prokaryotic cell. A cell can be a bacterial cell or can be derived from a bacterial cell. A cell can be an archaeal cell or derived from an archaeal cell. A cell can be a eukaryotic cell or derived from a eukaryotic cell. A cell can be a plant cell or derived from a plant cell. A cell can be an animal cell or derived from an animal cell. A cell can be an invertebrate cell or derived from an invertebrate cell. A cell can be a vertebrate cell or derived from a vertebrate cell. A cell can be a mammalian cell or derived from a mammalian cell. A cell can be a rodent cell or derived from a rodent cell. A cell can be a human cell or derived from a human cell. A cell can be a microbe cell or derived from a microbe cell. A cell can be a fungi cell or derived from a fungi cell. A cell can be an insect cell. A cell can be an arthropod cell. A cell can be a protozoan cell. A cell can be a helminth cell.

[00257] Suitable cells include a stem cell (e.g. an embryonic stem (ES) cell, an induced pluripotent stem (iPS) cell; a germ cell (e.g., an oocyte, a sperm, an oogonia, a spermatogonia, etc.); a somatic cell, e.g. a fibroblast, an oligodendrocyte, a glial cell, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, etc.

- [00258]** Suitable cells include human embryonic stem cells, fetal cardiomyocytes, myofibroblasts, mesenchymal stem cells, cardiomyocytes, adipocytes, totipotent cells, pluripotent cells, blood stem cells, myoblasts, adult stem cells, bone marrow cells, mesenchymal cells, embryonic stem cells, parenchymal cells, epithelial cells, endothelial cells, mesothelial cells, fibroblasts, osteoblasts, chondrocytes, exogenous cells, endogenous cells, stem cells, hematopoietic stem cells, bone-marrow derived progenitor cells, myocardial cells, skeletal cells, fetal cells, undifferentiated cells, multi-potent progenitor cells, unipotent progenitor cells, monocytes, cardiac myoblasts, skeletal myoblasts, macrophages, capillary endothelial cells, xenogenic cells, allogenic cells, and post-natal stem cells.
- [00259]** In some cases, the cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell. In some cases, the immune cell is a T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, or a macrophage. In some cases, the immune cell is a cytotoxic T cell. In some cases, the immune cell is a helper T cell. In some cases, the immune cell is a regulatory T cell (Treg). In some cases, the immune cell is a T effector cell.
- [00260]** In some cases, the cell is a stem cell. Stem cells include adult stem cells. Adult stem cells are also referred to as somatic stem cells.
- [00261]** Adult stem cells are resident in differentiated tissue, but retain the properties of self-renewal and ability to give rise to multiple cell types, usually cell types typical of the tissue in which the stem cells are found. Numerous examples of somatic stem cells are known to those of skill in the art, including muscle stem cells; hematopoietic stem cells; epithelial stem cells; neural stem cells; mesenchymal stem cells; mammary stem cells; intestinal stem cells; mesodermal stem cells; endothelial stem cells; olfactory stem cells; neural crest stem cells; and the like.
- [00262]** Stem cells of interest include mammalian stem cells, where the term “mammalian” refers to any animal classified as a mammal, including humans; non-human primates; domestic and farm animals; and zoo, laboratory, sports, or pet animals, such as dogs, horses, cats, cows, mice, rats, rabbits, etc. In some cases, the stem cell is a human stem cell. In some cases, the stem cell is a rodent (e.g., a mouse; a rat) stem cell. In some cases, the stem cell is a non-human primate stem cell.
- [00263]** Stem cells can express one or more stem cell markers, e.g., SOX9, KRT19, KRT7, LGR5, CA9, FXYD2, CDH6, CLDN18, TSPAN8, BPIFB1, OLFM4, CDH17, and PPARGC1A.
- [00264]** In some embodiments, the stem cell is a hematopoietic stem cell (HSC). HSCs are mesoderm-derived cells that can be isolated from bone marrow, blood, cord blood, fetal liver and yolk sac. HSCs are characterized as CD34⁺ and CD3⁻. HSCs can repopulate the erythroid,

neutrophil-macrophage, megakaryocyte and lymphoid hematopoietic cell lineages *in vivo*. *In vitro*, HSCs can be induced to undergo at least some self-renewing cell divisions and can be induced to differentiate to the same lineages as is seen *in vivo*. As such, HSCs can be induced to differentiate into one or more of erythroid cells, megakaryocytes, neutrophils, macrophages, and lymphoid cells.

[00265] In other embodiments, the stem cell is a neural stem cell (NSC). Neural stem cells (NSCs) are capable of differentiating into neurons, and glia (including oligodendrocytes, and astrocytes). A neural stem cell is a multipotent stem cell which is capable of multiple divisions, and under specific conditions can produce daughter cells which are neural stem cells, or neural progenitor cells that can be neuroblasts or glioblasts, e.g., cells committed to become one or more types of neurons and glial cells respectively. Methods of obtaining NSCs are known in the art.

[00266] In other embodiments, the stem cell is a mesenchymal stem cell (MSC). MSCs originally derived from the embryonal mesoderm and isolated from adult bone marrow, can differentiate to form muscle, bone, cartilage, fat, marrow stroma, and tendon. Methods of isolating MSC are known in the art; and any known method can be used to obtain MSC. See, e.g., U.S. Pat. No. 5,736,396, which describes isolation of human MSC.

[00267] A cell is in some cases a plant cell. A plant cell can be a cell of a monocotyledon. A cell can be a cell of a dicotyledon.

[00268] In some cases, the cell is a plant cell. For example, the cell can be a cell of a major agricultural plant, e.g., Barley, Beans (Dry Edible), Canola, Corn, Cotton (Pima), Cotton (Upland), Flaxseed, Hay (Alfalfa), Hay (Non-Alfalfa), Oats, Peanuts, Rice, Sorghum, Soybeans, Sugarbeets, Sugarcane, Sunflowers (Oil), Sunflowers (Non-Oil), Sweet Potatoes, Tobacco (Burley), Tobacco (Flue-cured), Tomatoes, Wheat (Durum), Wheat (Spring), Wheat (Winter), and the like. As another example, the cell is a cell of a vegetable crops which include but are not limited to, e.g., alfalfa sprouts, aloe leaves, arrow root, arrowhead, artichokes, asparagus, bamboo shoots, banana flowers, bean sprouts, beans, beet tops, beets, bittermelon, bok choy, broccoli, broccoli rabe (rappini), brussels sprouts, cabbage, cabbage sprouts, cactus leaf (nopales), calabaza, cardoon, carrots, cauliflower, celery, chayote, chinese artichoke (crosnes), chinese cabbage, chinese celery, chinese chives, choy sum, chrysanthemum leaves (tung ho), collard greens, corn stalks, corn-sweet, cucumbers, daikon, dandelion greens, dasheen, dau mue (pea tips), donqua (winter melon), eggplant, endive, escarole, fiddle head ferns, field cress, frisee, gai choy (chinese mustard), gailon, galanga (siam, thai ginger), garlic, ginger root, gobo, greens, hanover salad greens, huauzontle, jerusalem artichokes, jicama, kale greens, kohlrabi, lamb's quarters (quilete), lettuce (bibb), lettuce (boston), lettuce (boston red), lettuce (green leaf),

lettuce (iceberg), lettuce (lolla rossa), lettuce (oak leaf - green), lettuce (oak leaf - red), lettuce (processed), lettuce (red leaf), lettuce (romaine), lettuce (ruby romaine), lettuce (russian red mustard), linkok, lo bok, long beans, lotus root, mache, maguey (agave) leaves, malanga, mesculin mix, mizuna, moap (smooth luffa), moo, moqua (fuzzy squash), mushrooms, mustard, nagaimo, okra, ong choy, onions green, opo (long squash), ornamental corn, ornamental gourds, parsley, parsnips, peas, peppers (bell type), peppers, pumpkins, radicchio, radish sprouts, radishes, rape greens, rape greens, rhubarb, romaine (baby red), rutabagas, salicornia (sea bean), sinqua (angled/ridged luffa), spinach, squash, straw bales, sugarcane, sweet potatoes, swiss chard, tamarindo, taro, taro leaf, taro shoots, tatsoi, tepeguaje (guaje), tindora, tomatillos, tomatoes, tomatoes (cherry), tomatoes (grape type), tomatoes (plum type), tumeric, turnip tops greens, turnips, water chestnuts, yampi, yams (names), yu choy, yuca (cassava), and the like.

[00269] A cell is in some cases an arthropod cell. For example, the cell can be a cell of a sub-order, a family, a sub-family, a group, a sub-group, or a species of, e.g., *Chelicerata*, *Myriapodia*, *Hexipodia*, *Arachnida*, *Insecta*, *Archaeognatha*, *Thysanura*, *Palaeoptera*, *Ephemeroptera*, *Odonata*, *Anisoptera*, *Zygoptera*, *Neoptera*, *Exopterygota*, *Plecoptera*, *Embioptera*, *Orthoptera*, *Zoraptera*, *Dermaptera*, *Dictyoptera*, *Notoptera*, *Grylloblattidae*, *Mantophasmatidae*, *Phasmatodea*, *Blattaria*, *Isoptera*, *Mantodea*, *Parapneuroptera*, *Psocoptera*, *Thysanoptera*, *Phthiraptera*, *Hemiptera*, *Endopterygota* or *Holometabola*, *Hymenoptera*, *Coleoptera*, *Strepsiptera*, *Raphidioptera*, *Megaloptera*, *Neuroptera*, *Mecoptera*, *Siphonaptera*, *Diptera*, *Trichoptera*, or *Lepidoptera*.

[00270] A cell is in some cases an insect cell. For example, in some cases, the cell is a cell of a mosquito, a grasshopper, a true bug, a fly, a flea, a bee, a wasp, an ant, a louse, a moth, or a beetle.

KITS

[00271] The present disclosure provides a kit comprising a system of the present disclosure, or a component of a system of the present disclosure.

[00272] A kit of the present disclosure can comprise: a) an RNA-guided effector polypeptide of the present disclosure and a guide RNA; b) a fusion polypeptide of the present disclosure and a guide RNA; c) a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; d) an mRNA encoding an RNA-guided effector polypeptide of the present disclosure; and a guide RNA; e) an mRNA encoding a fusion polypeptide of the present disclosure; and a guide RNA; f) an mRNA encoding a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; g) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; h) a recombinant expression vector

comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; i) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a guide RNA, and a nucleotide sequence encoding a donor template nucleic acid; j) a first recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; k) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; l) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; and a donor template nucleic acid; m) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or n) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or some variation of one of (a) through (n).

[00273] In some cases, a kit of the present disclosure comprises: a) a RNA-guided effector polypeptide of the present disclosure, or the fusion polypeptide of the present disclosure; and b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide. In some cases, the guide RNA is a single-molecule guide RNA. In some cases, the RNA-guided effector polypeptide and the guide RNA are in separate containers. In some cases, the RNA-guided effector polypeptide and the guide RNA are together in a single container. In some cases, the guide RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, a Peptide Nucleic Acid, and a deoxyribonucleotide. In some cases, the kit further comprises a donor nucleic acid template.

[00274] A kit of the present disclosure can comprise: a) a component, as described above, of a system of the present disclosure, or can comprise a system of the present disclosure; and b) one or more additional reagents, e.g., i) a buffer; ii) a protease inhibitor; iii) a nuclease inhibitor; iv) a reagent required to develop or visualize a detectable label; v) a positive and/or negative control target DNA; vi) a positive and/or negative control guide RNA; and the like. A kit of the present

disclosure can comprise: a) a component, as described above, of a system of the present disclosure, or can comprise a system of the present disclosure; and b) a therapeutic agent.

[00275] A kit of the present disclosure can comprise a recombinant expression vector comprising: a) an insertion site for inserting a nucleic acid comprising a nucleotide sequence encoding a portion of a guide RNA that hybridizes to a target nucleotide sequence in a target nucleic acid; and b) a nucleotide sequence encoding the RNA-guided effector protein-binding portion of a guide RNA. A kit of the present disclosure can comprise a recombinant expression vector comprising: a) an insertion site for inserting a nucleic acid comprising a nucleotide sequence encoding a portion of a guide RNA that hybridizes to a target nucleotide sequence in a target nucleic acid; b) a nucleotide sequence encoding the RNA-guided effector protein-binding portion of a guide RNA; and c) a nucleotide sequence encoding RNA-guided effector polypeptide of the present disclosure.

[00276] A kit of the present disclosure can comprise a recombinant expression vector comprising: i) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; and ii) an insertion site for a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest. The insertion site can be located relative to the nucleotide sequence encoding the RNA-guided effector polypeptide, such that, after insertion of a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest, the resulting fusion polypeptide would comprise, in order from N-terminus to C-terminus: i) the RNA-guided effector polypeptide; and ii) the fusion partner. The insertion site can be located relative to the nucleotide sequence encoding the RNA-guided effector polypeptide, such that, after insertion of a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest, the resulting fusion polypeptide would comprise, in order from N-terminus to C-terminus: i) the fusion partner; and ii) the RNA-guided effector polypeptide. A kit of the present disclosure can comprise a recombinant expression vector comprising: i) a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; ii) an insertion site for a nucleic acid comprising a nucleotide sequence encoding a heterologous fusion partner of interest; iii) an insertion site for inserting a nucleic acid comprising a nucleotide sequence encoding a portion of a guide RNA that hybridizes to a target nucleotide sequence in a target nucleic acid; and iv) a nucleotide sequence encoding the RNA-guided effector protein-binding portion of a guide RNA

UTILITY

[00277] An RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure, finds use in a variety of methods (e.g., in combination with a guide RNA and in some cases further in combination with a donor template). An RNA-guided effector

polypeptide of the present disclosure can be used to bind to a target nucleic acid. For example, a fusion polypeptide of the present disclosure can be used to (i) modify (e.g., cleave, e.g., nick; methylate; etc.) target nucleic acid (DNA or RNA; single stranded or double stranded); (ii) modulate transcription of a target nucleic acid; (iii) label a target nucleic acid; (iv) bind a target nucleic acid (e.g., for purposes of isolation, labeling, imaging, tracking, etc.); (v) modify a polypeptide (e.g., a histone) associated with a target nucleic acid; and the like. Thus, the present disclosure provides a method of modifying a target nucleic acid. In some cases, a method of the present disclosure for modifying a target nucleic acid comprises contacting the target nucleic acid with: a) a fusion polypeptide of the present disclosure; and b) one or more (e.g., two) guide RNAs. In some cases, a method of the present disclosure for modifying a target nucleic acid comprises contacting the target nucleic acid with: a) a fusion polypeptide of the present disclosure; b) a guide RNA; and c) a donor nucleic acid (e.g., a donor template). In some cases, the contacting step is carried out in a cell *in vitro*. In some cases, the contacting step is carried out in a cell *in vivo*. In some cases, the contacting step is carried out in a cell *ex vivo*.

[00278] In some cases, a method of the present disclosure is a method of binding to a target nucleic acid. Because a method that uses an RNA-guided effector polypeptide includes binding of the RNA-guided effector polypeptide to a particular region in a target nucleic acid (by virtue of being targeted there by an associated guide RNA), the methods are generally referred to herein as methods of binding (e.g., a method of binding a target nucleic acid). However, it is to be understood that in some cases, while a method of binding may result in nothing more than binding of the target nucleic acid, in other cases, the method can have different final results (e.g., the method can result in modification of the target nucleic acid, e.g., cleavage/methylation/etc., modulation of transcription from the target nucleic acid; modulation of translation of the target nucleic acid; genome editing; modulation of a protein associated with the target nucleic acid; isolation of the target nucleic acid; etc.).

[00279] The present disclosure provides a method of binding and/or modifying a target nucleic acid and/or modifying a polypeptide that binds to a target nucleic acid, the method comprising contacting the target nucleic acid with: i) an RNA-guided effector polypeptide of the present disclosure; or ii) a fusion polypeptide of the present disclosure. In some cases, the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA. In some cases, the contacting results in genome editing. In some cases, the contacting takes place outside of a bacterial cell and outside of an archaeal cell. In some cases, the contacting takes place *in vitro* outside of a cell. In some cases, the contacting takes place inside of a target cell. In some cases, the target cell is a eukaryotic cell. In some cases, the target cell is *in vivo*. In some cases, the target cell is *ex vivo*. In some cases, the eukaryotic cell is

selected from the group consisting of: a plant cell, a fungal cell, a single cell eukaryotic organism, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell. In some cases, the method further comprises introducing a DNA donor template into the target cell.

[00280] For examples of suitable methods, see, for example, Jinek et al., *Science*. 2012 Aug 17;337(6096):816-21; Chylinski et al., *RNA Biol*. 2013 May;10(5):726-37; Ma et al., *Biomed Res Int*. 2013;2013:270805; Hou et al., *Proc Natl Acad Sci U S A*. 2013 Sep 24;110(39):15644-9; Jinek et al., *Elife*. 2013;2:e00471; Pattanayak et al., *Nat Biotechnol*. 2013 Sep;31(9):839-43; Qi et al., *Cell*. 2013 Feb 28;152(5):1173-83; Wang et al., *Cell*. 2013 May 9;153(4):910-8; Auer et al., *Genome Res*. 2013 Oct 31; Chen et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e19; Cheng et al., *Cell Res*. 2013 Oct;23(10):1163-71; Cho et al., *Genetics*. 2013 Nov;195(3):1177-80; DiCarlo et al., *Nucleic Acids Res*. 2013 Apr;41(7):4336-43; Dickinson et al., *Nat Methods*. 2013 Oct;10(10):1028-34; Ebina et al., *Sci Rep*. 2013;3:2510; Fujii et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e187; Hu et al., *Cell Res*. 2013 Nov;23(11):1322-5; Jiang et al., *Nucleic Acids Res*. 2013 Nov 1;41(20):e188; Larson et al., *Nat Protoc*. 2013 Nov;8(11):2180-96; Mali et. al., *Nat Methods*. 2013 Oct;10(10):957-63; Nakayama et al., *Genesis*. 2013 Dec;51(12):835-43; Ran et al., *Nat Protoc*. 2013 Nov;8(11):2281-308; Ran et al., *Cell*. 2013 Sep 12;154(6):1380-9; Upadhyay et al., *G3 (Bethesda)*. 2013 Dec 9;3(12):2233-8; Walsh et al., *Proc Natl Acad Sci U S A*. 2013 Sep 24;110(39):15514-5; Xie et al., *Mol Plant*. 2013 Oct 9; Yang et al., *Cell*. 2013 Sep 12;154(6):1370-9; and U.S. patents and patent applications: 8,906,616; 8,895,308; 8,889,418; 8,889,356; 8,871,445; 8,865,406; 8,795,965; 8,771,945; 8,697,359; 20140068797; 20140170753; 20140179006; 20140179770; 20140186843; 20140186919; 20140186958; 20140189896; 20140227787; 20140234972; 20140242664; 20140242699; 20140242700; 20140242702; 20140248702; 20140256046; 20140273037; 20140273226; 20140273230; 20140273231; 20140273232; 20140273233; 20140273234; 20140273235; 20140287938; 20140295556; 20140295557; 20140298547; 20140304853; 20140309487; 20140310828; 20140310830; 20140315985; 20140335063; 20140335620; 20140342456; 20140342457; 20140342458; 20140349400; 20140349405; 20140356867; 20140356956; 20140356958; 20140356959; 20140357523; 20140357530; 20140364333; and 20140377868; each of which is hereby incorporated by reference in its entirety.

[00281] For example, where a method of the present disclosure comprises contacting a target nucleic acid with a fusion polypeptide of the present disclosure complexed with a guide RNA, the present disclosure provides (but is not limited to) methods of cleaving a target nucleic acid; methods of editing a target nucleic acid; methods of modulating transcription from a target

nucleic acid; methods of isolating a target nucleic acid, methods of binding a target nucleic acid, methods of imaging a target nucleic acid, methods of modifying a target nucleic acid, and the like.

[00282] As used herein, the terms/phrases “contact a target nucleic acid” and “contacting a target nucleic acid”, for example, with an RNA-guided effector polypeptide or with a fusion polypeptide, etc., encompass all methods for contacting the target nucleic acid. For example, an RNA-guided effector polypeptide can be provided to a cell as protein, RNA (encoding the RNA-guided effector polypeptide), or DNA (encoding the RNA-guided effector polypeptide); while a guide RNA can be provided as a guide RNA or as a nucleic acid encoding the guide RNA. As another example, a fusion polypeptide can be provided to a cell as protein, RNA (encoding the fusion polypeptide), or DNA (encoding the fusion polypeptide); while a guide RNA can be provided as a guide RNA or as a nucleic acid encoding the guide RNA. As such, when, for example, performing a method in a cell (e.g., inside of a cell *in vitro*, inside of a cell *in vivo*, inside of a cell *ex vivo*), a method that includes contacting the target nucleic acid encompasses the introduction into the cell of any or all of the components in their active/final state (e.g., in the form of a protein(s) for an RNA-guided effector polypeptide; in the form of a protein for a fusion polypeptide; in the form of an RNA in some cases for the guide RNA), and also encompasses the introduction into the cell of one or more nucleic acids encoding one or more of the components (e.g., nucleic acid(s) comprising nucleotide sequence(s) encoding RNA-guided effector polypeptide or a fusion polypeptide, nucleic acid(s) comprising nucleotide sequence(s) encoding guide RNA(s), nucleic acid comprising a nucleotide sequence encoding a donor template, and the like). Because the methods can also be performed *in vitro* outside of a cell, a method that includes contacting a target nucleic acid, (unless otherwise specified) encompasses contacting outside of a cell *in vitro*, inside of a cell *in vitro*, inside of a cell *in vivo*, inside of a cell *ex vivo*, etc.

[00283] In some cases, a method of the present disclosure for modifying a target nucleic acid comprises contacting a target nucleic acid with RNA-guided effector polypeptide of the present disclosure, or with a fusion polypeptide of the present disclosure. In some cases, a method of the present disclosure for modifying a target nucleic acid comprises contacting the target nucleic acid with a fusion polypeptide of the present disclosure and a guide RNA. In some cases, a method of the present disclosure for binding a target nucleic acid comprises contacting the target nucleic acid with an RNA-guided effector polypeptide of the present disclosure, and a guide RNA.

Target nucleic acids and target cells of interest

- [00284]** An RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure, when bound to a guide RNA, can bind to a target nucleic acid (e.g., in the case of an RNA-guided effector polypeptide of the present disclosure), and in some cases, can bind to and modify a target nucleic acid (e.g., in the case of a fusion polypeptide of the present disclosure). A target nucleic acid can be any nucleic acid (e.g., DNA, RNA), can be double stranded or single stranded, can be any type of nucleic acid (e.g., a chromosome (genomic DNA), derived from a chromosome, chromosomal DNA, plasmid, viral, extracellular, intracellular, mitochondrial, chloroplast, linear, circular, etc.) and can be from any organism (e.g., as long as the guide RNA comprises a nucleotide sequence that hybridizes to a target sequence in a target nucleic acid, such that the target nucleic acid can be targeted).
- [00285]** A target nucleic acid can be DNA or RNA. A target nucleic acid can be double stranded (e.g., dsDNA, dsRNA) or single stranded (e.g., ssRNA, ssDNA). In some cases, a target nucleic acid is single stranded. In some cases, a target nucleic acid is a single stranded RNA (ssRNA). In some cases, a target ssRNA (e.g., a target cell ssRNA, a viral ssRNA, etc.) is selected from: mRNA, rRNA, tRNA, non-coding RNA (ncRNA), long non-coding RNA (lncRNA), and microRNA (miRNA). In some cases, a target nucleic acid is a single stranded DNA (ssDNA) (e.g., a viral DNA). As noted above, in some cases, a target nucleic acid is single stranded.
- [00286]** A target nucleic acid can be located anywhere, for example, outside of a cell in vitro, inside of a cell in vitro, inside of a cell in vivo, inside of a cell ex vivo. Suitable target cells (which can comprise target nucleic acids such as genomic DNA) include, but are not limited to: a bacterial cell; an archaeal cell; a cell of a single-cell eukaryotic organism; a plant cell; an algal cell, e.g., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens*, *C. agardh*, and the like; a fungal cell (e.g., a yeast cell); an animal cell; a cell from an invertebrate animal (e.g. fruit fly, a cnidarian, an echinoderm, a nematode, etc.); a cell of an insect (e.g., a mosquito; a bee; an agricultural pest; etc.); a cell of an arachnid (e.g., a spider; a tick; etc.); a cell from a vertebrate animal (e.g., a fish, an amphibian, a reptile, a bird, a mammal); a cell from a mammal (e.g., a cell from a rodent; a cell from a human; a cell of a non-human mammal; a cell of a rodent (e.g., a mouse, a rat); a cell of a lagomorph (e.g., a rabbit); a cell of an ungulate (e.g., a cow, a horse, a camel, a llama, a vicuña, a sheep, a goat, etc.); a cell of a marine mammal (e.g., a whale, a seal, an elephant seal, a dolphin, a sea lion; etc.) and the like. Any type of cell may be of interest (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced pluripotent stem (iPS) cell, a germ cell (e.g., an oocyte, a sperm, an oogonia, a spermatogonia, etc.), an adult stem cell, a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell; an in

vitro or in vivo embryonic cell of an embryo at any stage, e.g., a 1-cell, 2-cell, 4-cell, 8-cell, etc. stage zebrafish embryo; etc.).

[00287] Cells may be from established cell lines or they may be primary cells, where “primary cells”, “primary cell lines”, and “primary cultures” are used interchangeably herein to refer to cells and cells cultures that have been derived from a subject and allowed to grow in vitro for a limited number of passages, i.e. splittings, of the culture. For example, primary cultures are cultures that may have been passaged 0 times, 1 time, 2 times, 4 times, 5 times, 10 times, or 15 times, but not enough times go through the crisis stage. Typically, the primary cell lines are maintained for fewer than 10 passages in vitro. Target cells can be unicellular organisms and/or can be grown in culture. If the cells are primary cells, they may be harvest from an individual by any convenient method. For example, leukocytes may be conveniently harvested by apheresis, leukocytapheresis, density gradient separation, etc., while cells from tissues such as skin, muscle, bone marrow, spleen, liver, pancreas, lung, intestine, stomach, etc. can be conveniently harvested by biopsy.

[00288] In some of the above applications, the subject methods may be employed to induce target nucleic acid cleavage, target nucleic acid modification, and/or to bind target nucleic acids (e.g., for visualization, for collecting and/or analyzing, etc.) in mitotic or post-mitotic cells in vivo and/or ex vivo and/or in vitro (e.g., to disrupt production of a protein encoded by a targeted mRNA, to cleave or otherwise modify target DNA, to genetically modify a target cell, and the like). Because the guide RNA provides specificity by hybridizing to target nucleic acid, a mitotic and/or post-mitotic cell of interest in the disclosed methods may include a cell from any organism (e.g. a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a plant cell, an algal cell, e.g., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens*, *C. agardh*, and the like, a fungal cell (e.g., a yeast cell), an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human, etc.). In some cases, a subject RNA-guided effector protein (and/or nucleic acid encoding the protein such as DNA and/or RNA), and/or guide RNA (and/or a DNA encoding the guide RNA), and/or donor template, or a fusion polypeptide of the present disclosure (or a nucleic acid encoding the fusion polypeptide) and/or guide RNA, and/or RNP and/or system can be introduced into an individual (i.e., the target cell can be *in vivo*) (e.g., a mammal, a rat, a mouse, a pig, a primate, a non-human primate, a human, etc.). In some case, such an administration can be for the purpose of treating and/or preventing a disease, e.g., by editing the genome of targeted cells.

[00289] Plant cells include cells of a monocotyledon, and cells of a dicotyledon. The cells can be root cells, leaf cells, cells of the xylem, cells of the phloem, cells of the cambium, apical meristem cells, parenchyma cells, collenchyma cells, sclerenchyma cells, and the like. Plant cells include cells of agricultural crops such as wheat, corn, rice, sorghum, millet, soybean, etc. Plant cells include cells of agricultural fruit and nut plants, e.g., plant that produce apricots, oranges, lemons, apples, plums, pears, almonds, etc.

[00290] Additional examples of target cells are listed above in the section titled “Modified cells.” Non-limiting examples of cells (target cells) include: a prokaryotic cell, eukaryotic cell, a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a protozoa cell, a cell from a plant (e.g., cells from plant crops, fruits, vegetables, grains, soy bean, corn, maize, wheat, seeds, tomatoes, rice, cassava, sugarcane, pumpkin, hay, potatoes, cotton, cannabis, tobacco, flowering plants, conifers, gymnosperms, angiosperms, ferns, clubmosses, hornworts, liverworts, mosses, dicotyledons, monocotyledons, etc.), an algal cell, (e.g., *Botryococcus braunii*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana*, *Chlorella pyrenoidosa*, *Sargassum patens*, *C. agardh*, and the like), seaweeds (e.g. kelp) a fungal cell (e.g., a yeast cell, a cell from a mushroom), an animal cell, a cell from an invertebrate animal (e.g., fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal (e.g., an ungulate (e.g., a pig, a cow, a goat, a sheep); a rodent (e.g., a rat, a mouse); a non-human primate; a human; a feline (e.g., a cat); a canine (e.g., a dog); etc.), and the like. In some cases, the cell is a cell that does not originate from a natural organism (e.g., the cell can be a synthetically made cell; also referred to as an artificial cell).

[00291] A cell can be an *in vitro* cell (e.g., established cultured cell line). A cell can be an *ex vivo* cell (cultured cell from an individual). A cell can be an *in vivo* cell (e.g., a cell in an individual). A cell can be an isolated cell. A cell can be a cell inside of an organism. A cell can be an organism. A cell can be a cell in a cell culture (e.g., *in vitro* cell culture). A cell can be one of a collection of cells. A cell can be a prokaryotic cell or derived from a prokaryotic cell. A cell can be a bacterial cell or can be derived from a bacterial cell. A cell can be an archaeal cell or derived from an archaeal cell. A cell can be a eukaryotic cell or derived from a eukaryotic cell. A cell can be a plant cell or derived from a plant cell. A cell can be an animal cell or derived from an animal cell. A cell can be an invertebrate cell or derived from an invertebrate cell. A cell can be a vertebrate cell or derived from a vertebrate cell. A cell can be a mammalian cell or derived from a mammalian cell. A cell can be a rodent cell or derived from a rodent cell. A cell can be a human cell or derived from a human cell. A cell can be a microbe cell or derived from a microbe cell. A cell can be a fungi cell or derived from a fungi cell. A cell can be an insect cell. A cell can be an arthropod cell. A cell can be a protozoan cell. A cell can be a helminth cell.

- [00292]** Suitable cells include a stem cell (e.g. an embryonic stem (ES) cell, an induced pluripotent stem (iPS) cell; a germ cell (e.g., an oocyte, a sperm, an oogonia, a spermatogonia, etc.); a somatic cell, e.g. a fibroblast, an oligodendrocyte, a glial cell, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell, etc.
- [00293]** Suitable cells include human embryonic stem cells, fetal cardiomyocytes, myofibroblasts, mesenchymal stem cells, cardiomyocytes, adipocytes, totipotent cells, pluripotent cells, blood stem cells, myoblasts, adult stem cells, bone marrow cells, mesenchymal cells, embryonic stem cells, parenchymal cells, epithelial cells, endothelial cells, mesothelial cells, fibroblasts, osteoblasts, chondrocytes, exogenous cells, endogenous cells, stem cells, hematopoietic stem cells, bone-marrow derived progenitor cells, myocardial cells, skeletal cells, fetal cells, undifferentiated cells, multi-potent progenitor cells, unipotent progenitor cells, monocytes, cardiac myoblasts, skeletal myoblasts, macrophages, capillary endothelial cells, xenogenic cells, allogenic cells, and post-natal stem cells.
- [00294]** In some cases, the cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell. In some cases, the immune cell is a T cell, a B cell, a monocyte, a natural killer cell, a dendritic cell, or a macrophage. In some cases, the immune cell is a cytotoxic T cell. In some cases, the immune cell is a helper T cell. In some cases, the immune cell is a regulatory T cell (Treg).
- [00295]** In some cases, the cell is a stem cell. Stem cells include adult stem cells. Adult stem cells are also referred to as somatic stem cells.
- [00296]** Adult stem cells are resident in differentiated tissue, but retain the properties of self-renewal and ability to give rise to multiple cell types, usually cell types typical of the tissue in which the stem cells are found. Numerous examples of somatic stem cells are known to those of skill in the art, including muscle stem cells; hematopoietic stem cells; epithelial stem cells; neural stem cells; mesenchymal stem cells; mammary stem cells; intestinal stem cells; mesodermal stem cells; endothelial stem cells; olfactory stem cells; neural crest stem cells; and the like.
- [00297]** Stem cells of interest include mammalian stem cells, where the term “mammalian” refers to any animal classified as a mammal, including humans; non-human primates; domestic and farm animals; and zoo, laboratory, sports, or pet animals, such as dogs, horses, cats, cows, mice, rats, rabbits, etc. In some cases, the stem cell is a human stem cell. In some cases, the stem cell is a rodent (e.g., a mouse; a rat) stem cell. In some cases, the stem cell is a non-human primate stem cell.

- [00298]** Stem cells can express one or more stem cell markers, e.g., SOX9, KRT19, KRT7, LGR5, CA9, FXYD2, CDH6, CLDN18, TSPAN8, BPIFB1, OLFM4, CDH17, and PPARGC1A.
- [00299]** In some embodiments, the stem cell is a hematopoietic stem cell (HSC). HSCs are mesoderm-derived cells that can be isolated from bone marrow, blood, cord blood, fetal liver and yolk sac. HSCs are characterized as CD34+ and CD3-. HSCs can repopulate the erythroid, neutrophil-macrophage, megakaryocyte and lymphoid hematopoietic cell lineages in vivo. In vitro, HSCs can be induced to undergo at least some self-renewing cell divisions and can be induced to differentiate to the same lineages as is seen in vivo. As such, HSCs can be induced to differentiate into one or more of erythroid cells, megakaryocytes, neutrophils, macrophages, and lymphoid cells.
- [00300]** In other embodiments, the stem cell is a neural stem cell (NSC). Neural stem cells (NSCs) are capable of differentiating into neurons, and glia (including oligodendrocytes, and astrocytes). A neural stem cell is a multipotent stem cell which is capable of multiple divisions, and under specific conditions can produce daughter cells which are neural stem cells, or neural progenitor cells that can be neuroblasts or glioblasts, e.g., cells committed to become one or more types of neurons and glial cells respectively. Methods of obtaining NSCs are known in the art.
- [00301]** In other embodiments, the stem cell is a mesenchymal stem cell (MSC). MSCs originally derived from the embryonal mesoderm and isolated from adult bone marrow, can differentiate to form muscle, bone, cartilage, fat, marrow stroma, and tendon. Methods of isolating MSC are known in the art; and any known method can be used to obtain MSC. See, e.g., U.S. Pat. No. 5,736,396, which describes isolation of human MSC.
- [00302]** A cell is in some cases a plant cell. A plant cell can be a cell of a monocotyledon. A cell can be a cell of a dicotyledon.
- [00303]** In some cases, the cell is a plant cell. For example, the cell can be a cell of a major agricultural plant, e.g., Barley, Beans (Dry Edible), Canola, Corn, Cotton (Pima), Cotton (Upland), Flaxseed, Hay (Alfalfa), Hay (Non-Alfalfa), Oats, Peanuts, Rice, Sorghum, Soybeans, Sugarbeets, Sugarcane, Sunflowers (Oil), Sunflowers (Non-Oil), Sweet Potatoes, Tobacco (Burley), Tobacco (Flue-cured), Tomatoes, Wheat (Durum), Wheat (Spring), Wheat (Winter), and the like. As another example, the cell is a cell of a vegetable crops which include but are not limited to, e.g., alfalfa sprouts, aloe leaves, arrow root, arrowhead, artichokes, asparagus, bamboo shoots, banana flowers, bean sprouts, beans, beet tops, beets, bittermelon, bok choy, broccoli, broccoli rabe (rappini), brussels sprouts, cabbage, cabbage sprouts, cactus leaf (nopales), calabaza, cardoon, carrots, cauliflower, celery, chayote, chinese artichoke (crosnes),

chinese cabbage, chinese celery, chinese chives, choy sum, chrysanthemum leaves (tung ho), collard greens, corn stalks, corn-sweet, cucumbers, daikon, dandelion greens, dasheen, dau mue (pea tips), donqua (winter melon), eggplant, endive, escarole, fiddle head ferns, field cress, frisee, gai choy (chinese mustard), gailon, galanga (siam, thai ginger), garlic, ginger root, gobo, greens, hanover salad greens, huauzontle, jerusalem artichokes, jicama, kale greens, kohlrabi, lamb's quarters (quilete), lettuce (bibb), lettuce (boston), lettuce (boston red), lettuce (green leaf), lettuce (iceberg), lettuce (lolla rossa), lettuce (oak leaf - green), lettuce (oak leaf - red), lettuce (processed), lettuce (red leaf), lettuce (romaine), lettuce (ruby romaine), lettuce (russian red mustard), linkok, lo bok, long beans, lotus root, mache, maguey (agave) leaves, malanga, mesculin mix, mizuna, moap (smooth luffa), moo, moqua (fuzzy squash), mushrooms, mustard, nagaimo, okra, ong choy, onions green, opo (long squash), ornamental corn, ornamental gourds, parsley, parsnips, peas, peppers (bell type), peppers, pumpkins, radicchio, radish sprouts, radishes, rape greens, rape greens, rhubarb, romaine (baby red), rutabagas, salicornia (sea bean), sinqua (angled/ridged luffa), spinach, squash, straw bales, sugarcane, sweet potatoes, swiss chard, tamarindo, taro, taro leaf, taro shoots, tatsoi, tepeguaje (guaje), tindora, tomatillos, tomatoes, tomatoes (cherry), tomatoes (grape type), tomatoes (plum type), tumeric, turnip tops greens, turnips, water chestnuts, yampi, yams (names), yu choy, yuca (cassava), and the like.

[00304] A cell is in some cases an arthropod cell. For example, the cell can be a cell of a sub-order, a family, a sub-family, a group, a sub-group, or a species of, e.g., Chelicerata, Myriapodia, Hexipodia, Arachnida, Insecta, Archaeognatha, Thysanura, Palaeoptera, Ephemeroptera, Odonata, Anisoptera, Zygoptera, Neoptera, Exopterygota, Plecoptera, Embioptera, Orthoptera, Zoraptera, Dermaptera, Dictyoptera, Notoptera, Grylloblattidae, Mantophasmatidae, Phasmatodea, Blattaria, Isoptera, Mantodea, Parapneuroptera, Psocoptera, Thysanoptera, Phthiraptera, Hemiptera, Endopterygota or Holometabola, Hymenoptera, Coleoptera, Strepsiptera, Raphidioptera, Megaloptera, Neuroptera, Mecoptera, Siphonaptera, Diptera, Trichoptera, or Lepidoptera.

[00305] A cell is in some cases an insect cell. For example, in some cases, the cell is a cell of a mosquito, a grasshopper, a true bug, a fly, a flea, a bee, a wasp, an ant, a louse, a moth, or a beetle.

Introducing components into a target cell

[00306] A Cas9 guide RNA (or a nucleic acid comprising a nucleotide sequence encoding same), and/or a Cas9 fusion polypeptide (or a nucleic acid comprising a nucleotide sequence encoding same) and/or a donor polynucleotide can be introduced into a host cell by any of a variety of well-known methods.

[00307] Methods of introducing a nucleic acid into a cell are known in the art, and any convenient method can be used to introduce a nucleic acid (e.g., an expression construct) into a target cell (e.g., eukaryotic cell, human cell, stem cell, progenitor cell, and the like). Suitable methods are described in more detail elsewhere herein and include e.g., viral or bacteriophage infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro injection, nanoparticle-mediated nucleic acid delivery (see, e.g., Panyam et., al Adv Drug Deliv Rev. 2012 Sep 13. pii: S0169-409X(12)00283-9. doi: 10.1016/j.addr.2012.09.023), and the like. Any or all of the components can be introduced into a cell as a composition (e.g., including any convenient combination of: an RNA-guided effector polypeptide, a fusion polypeptide, a guide RNA, a donor polynucleotide, etc.) using known methods, e.g., such as nucleofection.

Donor Polynucleotide (donor template)

[00308] Guided by a dual or single guide RNA, a fusion protein in some cases generates site-specific double strand breaks (DSBs) or single strand breaks (SSBs) within double-stranded DNA (dsDNA) target nucleic acids, which are repaired either by non-homologous end joining (NHEJ) or homology-directed recombination (HDR).

[00309] In some cases, contacting a target DNA (with fusion protein and a guide RNA) occurs under conditions that are permissive for nonhomologous end joining or homology-directed repair. Thus, in some cases, a subject method includes contacting the target DNA with a donor polynucleotide (e.g., by introducing the donor polynucleotide into a cell), wherein the donor polynucleotide, a portion of the donor polynucleotide, a copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA. In some cases, the method does not comprise contacting a cell with a donor polynucleotide, and the target DNA is modified such that nucleotides within the target DNA are deleted.

[00310] In applications in which it is desirable to insert a polynucleotide sequence into the genome where a target sequence is cleaved, a donor polynucleotide (a nucleic acid comprising a donor sequence) can also be provided to the cell. By a “donor sequence” or “donor polynucleotide” or “donor template” it is meant a nucleic acid sequence to be inserted at the site cleaved by the fusion protein (e.g., after dsDNA cleavage, after nicking a target DNA, after dual nicking a target DNA, and the like). The donor polynucleotide can contain sufficient homology to a genomic sequence at the target site, e.g. 70%, 80%, 85%, 90%, 95%, or 100% homology with the nucleotide sequences flanking the target site, e.g. within about 50 bases or less of the target site, e.g. within about 30 bases, within about 15 bases, within about 10 bases, within about

5 bases, or immediately flanking the target site, to support homology-directed repair between it and the genomic sequence to which it bears homology. Approximately 25, 50, 100, or 200 nucleotides, or more than 200 nucleotides, of sequence homology between a donor and a genomic sequence (or any integral value between 10 and 200 nucleotides, or more) can support homology-directed repair. Donor polynucleotides can be of any length, e.g. 10 nucleotides or more, 50 nucleotides or more, 100 nucleotides or more, 250 nucleotides or more, 500 nucleotides or more, 1000 nucleotides or more, 5000 nucleotides or more, etc.

[00311] The donor sequence is typically not identical to the genomic sequence that it replaces. Rather, the donor sequence may contain at least one or more single base changes, insertions, deletions, inversions or rearrangements with respect to the genomic sequence, so long as sufficient homology is present to support homology-directed repair (e.g., for gene correction, e.g., to convert a disease-causing base pair to a non disease-causing base pair). In some embodiments, the donor sequence comprises a non-homologous sequence flanked by two regions of homology, such that homology-directed repair between the target DNA region and the two flanking sequences results in insertion of the non-homologous sequence at the target region. Donor sequences may also comprise a vector backbone containing sequences that are not homologous to the DNA region of interest and that are not intended for insertion into the DNA region of interest. Generally, the homologous region(s) of a donor sequence will have at least 50% sequence identity to a genomic sequence with which recombination is desired. In certain embodiments, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 99.9% sequence identity is present. Any value between 1% and 100% sequence identity can be present, depending upon the length of the donor polynucleotide.

[00312] The donor sequence may comprise certain sequence differences as compared to the genomic sequence, e.g. restriction sites, nucleotide polymorphisms, selectable markers (e.g., drug resistance genes, fluorescent proteins, enzymes etc.), etc., which may be used to assess for successful insertion of the donor sequence at the cleavage site or in some cases may be used for other purposes (e.g., to signify expression at the targeted genomic locus). In some cases, if located in a coding region, such nucleotide sequence differences will not change the amino acid sequence, or will make silent amino acid changes (i.e., changes which do not affect the structure or function of the protein). Alternatively, these sequences differences may include flanking recombination sequences such as FLPs, loxP sequences, or the like, that can be activated at a later time for removal of the marker sequence.

[00313] In some cases, the donor sequence is provided to the cell as single-stranded DNA. In some cases, the donor sequence is provided to the cell as double-stranded DNA. It may be introduced into a cell in linear or circular form. If introduced in linear form, the ends of the

donor sequence may be protected (e.g., from exonucleolytic degradation) by any convenient method and such methods are known to those of skill in the art. For example, one or more dideoxynucleotide residues can be added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides can be ligated to one or both ends. See, for example, Chang et al. (1987) Proc. Natl. Acad Sci USA 84:4959-4963; Nehls et al. (1996) Science 272:886-889. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues. As an alternative to protecting the termini of a linear donor sequence, additional lengths of sequence may be included outside of the regions of homology that can be degraded without impacting recombination. A donor sequence can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, donor sequences can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (e.g., adenovirus, AAV), as described elsewhere herein for nucleic acids encoding a guide RNA and/or a fusion polypeptide and/or donor polynucleotide.

TRANSGENIC, NON-HUMAN ORGANISMS

[00314] As described above, in some cases, a nucleic acid (e.g., a recombinant expression vector) of the present disclosure (e.g., a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure; etc.), is used as a transgene to generate a transgenic non-human organism that produces an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. The present disclosure provides a transgenic-non-human organism comprising a nucleotide sequence encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure.

Transgenic, non-human animals

[00315] The present disclosure provides a transgenic non-human animal, which animal comprises a transgene comprising a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide or a fusion polypeptide. In some embodiments, the genome of the transgenic non-human animal comprises a nucleotide sequence encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. In some cases, the transgenic non-human animal is homozygous for the genetic modification. In some cases, the transgenic non-human animal is heterozygous for the genetic modification. In some embodiments, the transgenic non-human animal is a vertebrate, for example, a fish (e.g., salmon,

trout, zebra fish, gold fish, puffer fish, cave fish, etc.), an amphibian (frog, newt, salamander, etc.), a bird (e.g., chicken, turkey, etc.), a reptile (e.g., snake, lizard, etc.), a non-human mammal (e.g., an ungulate, e.g., a pig, a cow, a goat, a sheep, etc.; a lagomorph (e.g., a rabbit); a rodent (e.g., a rat, a mouse); a non-human primate; etc.), etc. In some cases, the transgenic non-human animal is an invertebrate. In some cases, the transgenic non-human animal is an insect (e.g., a mosquito; an agricultural pest; etc.). In some cases, the transgenic non-human animal is an arachnid.

[00316] Nucleotide sequences encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure can be under the control of (i.e., operably linked to) an unknown promoter (e.g., when the nucleic acid randomly integrates into a host cell genome) or can be under the control of (i.e., operably linked to) a known promoter. Suitable known promoters can be any known promoter and include constitutively active promoters (e.g., CMV promoter), inducible promoters (e.g., heat shock promoter, tetracycline-regulated promoter, steroid-regulated promoter, metal-regulated promoter, estrogen receptor-regulated promoter, etc.), spatially restricted and/or temporally restricted promoters (e.g., a tissue specific promoter, a cell type specific promoter, etc.), etc.

Transgenic plants

[00317] As described above, in some cases, a nucleic acid (e.g., a recombinant expression vector) of the present disclosure (e.g., a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure; a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure; etc.), is used as a transgene to generate a transgenic plant that produces an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. The present disclosure provides a transgenic plant comprising a nucleotide sequence encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. In some embodiments, the genome of the transgenic plant comprises a subject nucleic acid. In some embodiments, the transgenic plant is homozygous for the genetic modification. In some embodiments, the transgenic plant is heterozygous for the genetic modification.

[00318] Methods of introducing exogenous nucleic acids into plant cells are well known in the art. Such plant cells are considered “transformed,” as defined above. Suitable methods include viral infection (such as double stranded DNA viruses), transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, silicon carbide whiskers technology, Agrobacterium-mediated transformation and the like. The choice of method is generally dependent on the type of cell being transformed

and the circumstances under which the transformation is taking place (i.e. in vitro, ex vivo, or in vivo).

[00319] Transformation methods based upon the soil bacterium *Agrobacterium tumefaciens* are particularly useful for introducing an exogenous nucleic acid molecule into a vascular plant. The wild type form of *Agrobacterium* contains a Ti (tumor-inducing) plasmid that directs production of tumorigenic crown gall growth on host plants. Transfer of the tumor-inducing T-DNA region of the Ti plasmid to a plant genome requires the Ti plasmid-encoded virulence genes as well as T-DNA borders, which are a set of direct DNA repeats that delineate the region to be transferred. An *Agrobacterium*-based vector is a modified form of a Ti plasmid, in which the tumor inducing functions are replaced by the nucleic acid sequence of interest to be introduced into the plant host.

[00320] *Agrobacterium*-mediated transformation generally employs cointegrate vectors or binary vector systems, in which the components of the Ti plasmid are divided between a helper vector, which resides permanently in the *Agrobacterium* host and carries the virulence genes, and a shuttle vector, which contains the gene of interest bounded by T-DNA sequences. A variety of binary vectors is well known in the art and are commercially available, for example, from Clontech (Palo Alto, Calif.). Methods of coculturing *Agrobacterium* with cultured plant cells or wounded tissue such as leaf tissue, root explants, hypocotyledons, stem pieces or tubers, for example, also are well known in the art. See, e.g., Glick and Thompson, (eds.), *Methods in Plant Molecular Biology and Biotechnology*, Boca Raton, Fla.: CRC Press (1993).

[00321] Microprojectile-mediated transformation also can be used to produce a subject transgenic plant. This method, first described by Klein et al. (*Nature* 327:70-73 (1987)), relies on microprojectiles such as gold or tungsten that are coated with the desired nucleic acid molecule by precipitation with calcium chloride, spermidine or polyethylene glycol. The microprojectile particles are accelerated at high speed into an angiosperm tissue using a device such as the BIOLISTIC PD-1000 (Biorad; Hercules Calif.).

[00322] A nucleic acid of the present disclosure (e.g., a nucleic acid (e.g., a recombinant expression vector) comprising a nucleotide sequence encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure) may be introduced into a plant in a manner such that the nucleic acid is able to enter a plant cell(s), e.g., via an in vivo or ex vivo protocol. By "in vivo," it is meant in the nucleic acid is administered to a living body of a plant e.g. infiltration. By "ex vivo" it is meant that cells or explants are modified outside of the plant, and then such cells or organs are regenerated to a plant. A number of vectors suitable for stable transformation of plant cells or for the establishment of transgenic plants have been described, including those described in Weissbach and Weissbach, (1989) *Methods for Plant Molecular*

Biology Academic Press, and Gelvin et al., (1990) Plant Molecular Biology Manual, Kluwer Academic Publishers. Specific examples include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed by Herrera-Estrella et al. (1983) Nature 303: 209, Bevan (1984) Nucl Acid Res. 12: 8711-8721, Klee (1985) Bio/Technolo 3: 637-642. Alternatively, non-Ti vectors can be used to transfer the DNA into plants and cells by using free DNA delivery techniques. By using these methods transgenic plants such as wheat, rice (Christou (1991) Bio/Technology 9:957-9 and 4462) and corn (Gordon-Kamm (1990) Plant Cell 2: 603-618) can be produced. An immature embryo can also be a good target tissue for monocots for direct DNA delivery techniques by using the particle gun (Weeks et al. (1993) Plant Physiol 102: 1077-1084; Vasil (1993) Bio/Technolo 10: 667-674; Wan and Lemeaux (1994) Plant Physiol 104: 37-48 and for *Agrobacterium*-mediated DNA transfer (Ishida et al. (1996) Nature Biotech 14: 745-750). Exemplary methods for introduction of DNA into chloroplasts are biolistic bombardment, polyethylene glycol transformation of protoplasts, and microinjection (Danieli et al Nat. Biotechnol 16:345-348, 1998; Staub et al Nat. Biotechnol 18: 333-338, 2000; O'Neill et al Plant J. 3:729-738, 1993; Knoblauch et al Nat. Biotechnol 17: 906-909; U.S. Pat. Nos. 5,451,513, 5,545,817, 5,545,818, and 5,576,198; in Intl. Application No. WO 95/16783; and in Boynton et al., Methods in Enzymology 217: 510-536 (1993), Svab et al., Proc. Natl. Acad. Sci. USA 90: 913-917 (1993), and McBride et al., Proc. Natl. Acad. Sci. USA 91: 7301-7305 (1994)). Any vector suitable for the methods of biolistic bombardment, polyethylene glycol transformation of protoplasts and microinjection will be suitable as a targeting vector for chloroplast transformation. Any double stranded DNA vector may be used as a transformation vector, especially when the method of introduction does not utilize *Agrobacterium*.

[00323] Plants which can be genetically modified include grains, forage crops, fruits, vegetables, oil seed crops, palms, forestry, and vines. Specific examples of plants which can be modified follow: maize, banana, peanut, field peas, sunflower, tomato, canola, tobacco, wheat, barley, oats, potato, soybeans, cotton, carnations, sorghum, lupin and rice.

[00324] The present disclosure provides transformed plant cells, tissues, plants and products that contain the transformed plant cells. A feature of the subject transformed cells, and tissues and products that include the same is the presence of a subject nucleic acid integrated into the genome, and production by plant cells of an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure. Recombinant plant cells of the present invention are useful as populations of recombinant cells, or as a tissue, seed, whole plant, stem, fruit, leaf, root, flower, stem, tuber, grain, animal feed, a field of plants, and the like.

[00325] Nucleotide sequences encoding an RNA-guided effector polypeptide, or a fusion polypeptide, of the present disclosure can be under the control of (i.e., operably linked to) an

unknown promoter (e.g., when the nucleic acid randomly integrates into a host cell genome) or can be under the control of (i.e., operably linked to) a known promoter. Suitable known promoters can be any known promoter and include constitutively active promoters, inducible promoters, spatially restricted and/or temporally restricted promoters, etc.

COMPOSITIONS, RIBONUCLEOPROTEIN COMPLEXES, AND SYSTEMS

[00326] The present disclosure provides a ribonucleoprotein (RNP) complex comprising: a) a fusion polypeptide of the present disclosure; and b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide component of the fusion polypeptide. The present disclosure provides an RNP complex comprising: a) an RNA-guided effector polypeptide of the present disclosure; and b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide. In some cases, the guide RNA is a single-molecule guide RNA.

[00327] The present disclosure provides a composition comprising: a) an RNP complex of the present disclosure; and b) at least one additional component, where the at least one additional component is selected from a salt, a buffer, a protease inhibitor, a nuclease inhibitor, a lipid, and the like. The present disclosure provides a composition comprising: a) an RNP complex of the present disclosure; and b) a pharmaceutically acceptable excipient. In some cases, a composition of the present disclosure comprises a lipid. In some cases, a composition of the present disclosure comprises a liposome, a hydrogel, a microparticle, a nanoparticle, or a block copolymer micelle.

[00328] The present disclosure provides a system comprising an RNA-guided effector polypeptide of the present disclosure, or a fusion polypeptide of the present disclosure. The present disclosure provides a system comprising a nucleic acid comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, or a nucleic acid comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure. In some cases, a system of the present disclosure further comprises a guide RNA.

[00329] A system of the present disclosure can comprise: a) an RNA-guided effector polypeptide of the present disclosure and a guide RNA; b) a fusion polypeptide of the present disclosure and a guide RNA; c) a fusion polypeptide of the present disclosure, a guide RNA, and a donor template nucleic acid; d) an mRNA encoding an RNA-guided effector polypeptide of the present disclosure; and a guide RNA; e) an mRNA encoding a fusion polypeptide of the present disclosure; and a guide RNA; f) an mRNA encoding a fusion polypeptide of the present

disclosure, a guide RNA, and a donor template nucleic acid; g) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; h) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure and a nucleotide sequence encoding a guide RNA; i) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a guide RNA, and a nucleotide sequence encoding a donor template nucleic acid; j) a first recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; k) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; l) a first recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, and a second recombinant expression vector comprising a nucleotide sequence encoding a guide RNA; and a donor template nucleic acid; m) a recombinant expression vector comprising a nucleotide sequence encoding an RNA-guided effector polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or n) a recombinant expression vector comprising a nucleotide sequence encoding a fusion polypeptide of the present disclosure, a nucleotide sequence encoding a first guide RNA, and a nucleotide sequence encoding a second guide RNA; or some variation of one of (a) through (n).

Examples of Non-Limiting Aspects of the Disclosure

[00330] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-77 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[00331] Aspect 1. An RNA-guided effector polypeptide having a length of from about 610 amino acids to about 1310 amino acids, wherein the RNA-guided effector polypeptide comprises one or more of:

[00332] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;

- [00333] ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
- [00334] iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- [00335] d) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112,
- [00336] wherein the amino acid numbering is based on SEQ ID NO:5, or corresponding amino acid positions of an amino acid sequence set forth in any one of SEQ ID NOs:6-816.
- [00337] Aspect 2. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324.
- [00338] Aspect 3. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739.
- [00339] Aspect 4. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.
- [00340] Aspect 5. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00341] Aspect 6. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00342] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324; and
- [00343] ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739.
- [00344] Aspect 7. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00345] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324; and
- [00346] ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.
- [00347] Aspect 8. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:

- [00348] i) a deletion of from 5 amino acids to 158 amino acids of amino acids 166-324; and
- [00349] ii) a deletion of from 20 amino acids to 118 amino acids of amino acids 994-1112.
- [00350] Aspect 9. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00351] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;
- [00352] ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and
- [00353] iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.
- [00354] Aspect 10. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00355] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;
- [00356] ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- [00357] iii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00358] Aspect 11. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00359] i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;
- [00360] ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
- [00361] iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- [00362] iv) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00363] Aspect 12. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00364] i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and
- [00365] ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.

- [00366] Aspect 13. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00367] i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and
- [00368] ii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00369] Aspect 14. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00370] i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
- [00371] ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- [00372] iii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00373] Aspect 15. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00374] i) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- [00375] ii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
- [00376] Aspect 16. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 179-296 based on the number of SEQ ID NO:5.
- [00377] Aspect 17. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 503-708 based on the number of SEQ ID NO:5.
- [00378] Aspect 18. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 792-897 based on the number of SEQ ID NO:5.
- [00379] Aspect 19. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.
- [00380] Aspect 20. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:

- [00381] i) a deletion of amino acids 503-708 based on the number of SEQ ID NO:5;
- [00382] ii) a deletion of amino acids 792-897 based on the number of SEQ ID NO:5; and
- [00383] iii) a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.
- [00384] Aspect 21. The RNA-guided effector polypeptide of aspect 1, wherein the RNA-guided effector polypeptide comprises:
- [00385] i) a deletion of amino acids 179-296 based on the number of SEQ ID NO:5;
- [00386] ii) a deletion of amino acids 503-708 based on the number of SEQ ID NO:5;
- [00387] iii) a deletion of amino acids 792-897 based on the number of SEQ ID NO:5; and
- [00388] iv) a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.
- [00389] Aspect 22. The RNA-guided effector polypeptide of aspect 1, wherein the polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in FIG. 2, wherein the RNA-guided effector polypeptide is 990 amino acids in length.
- [00390] Aspect 23. The RNA-guided effector polypeptide of aspect 1, wherein the polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in FIG. 3, wherein the RNA-guided effector polypeptide is 874 amino acids in length.
- [00391] Aspect 24. A nucleic acid comprising a nucleotide sequence encoding the RNA-guided effector polypeptide of any one of aspects 1-23.
- [00392] Aspect 25. The nucleic acid of aspect 24, wherein the nucleotide sequence is operably linked to a transcriptional control element.
- [00393] Aspect 26. The nucleic acid of aspect 25, wherein the transcriptional control element is a promoter.
- [00394] Aspect 27. The nucleic acid of aspect 26, wherein the promoter is a regulatable promoter.
- [00395] Aspect 28. The nucleic acid of aspect 27, wherein the promoter is functional in a eukaryotic cell.
- [00396] Aspect 29. The nucleic acid of any one of aspects 24-28, further comprising a nucleotide sequence encoding a guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.
- [00397] Aspect 30. A recombinant expression vector comprising the nucleic acid of any one of aspects 24-29.

- [00398] Aspect 31. The recombinant expression vector of aspect 30, wherein the recombinant expression vector is recombinant viral expression vector.
- [00399] Aspect 32. A fusion polypeptide comprising:
- [00400] a) the RNA-guided effector polypeptide of any one of aspects 1-23; and
- [00401] b) a heterologous fusion partner.
- [00402] Aspect 33. The fusion polypeptide of aspect 32, wherein the heterologous fusion partner provides for control of gene expression.
- [00403] Aspect 34. The fusion polypeptide of aspect 32, wherein the heterologous fusion partner is a protein-modifying enzyme.
- [00404] Aspect 35. The fusion polypeptide of aspect 32, wherein the heterologous fusion partner is a modifying enzyme is a DNA-modifying enzyme.
- [00405] Aspect 36. The fusion polypeptide of aspect 32, wherein the heterologous fusion partner exhibits nuclease activity.
- [00406] Aspect 37. The fusion polypeptide of aspect 36, wherein the nuclease activity is double-stranded DNA cleavage activity.
- [00407] Aspect 38. The fusion polypeptide of aspect 37, wherein the heterologous fusion partner comprises an amino acid sequence having at least 85% amino acid sequence identity to the catalytic domain of the amino acid sequence depicted in FIG. 2A and has a length of no more than 200 amino acids.
- [00408] Aspect 39. The fusion polypeptide of any one of aspects 32-38, wherein the fusion partner has a length of no more than 400 amino acids.
- [00409] Aspect 40. The fusion polypeptide of aspect 35, wherein the heterologous fusion partner is a base editor.
- [00410] Aspect 41. The fusion polypeptide of aspect 40, wherein the base editor is a cytidine deaminase.
- [00411] Aspect 42. The fusion polypeptide of aspect 40, wherein the base editor is an adenosine deaminase.
- [00412] Aspect 43. The fusion polypeptide of any one of aspects 32-42, further comprising one or more nuclear localization sequences (NLS).
- [00413] Aspect 44. The fusion polypeptide of aspect 43, wherein the fusion polypeptide comprises a single NLS.
- [00414] Aspect 45. The fusion polypeptide of aspect 43, wherein the fusion polypeptide comprises 2 or more NLSs.

- [00415] Aspect 46. The fusion polypeptide of aspect 43, wherein the fusion polypeptide comprises an NLS at the C-terminus, at the N-terminus, or at the C-terminus and the N-terminus, of the fusion polypeptide.
- [00416] Aspect 47. The fusion polypeptide of any one of aspects 32-42, further comprising an endosomolytic peptide.
- [00417] Aspect 48. A nucleic acid comprising a nucleotide sequence encoding the fusion polypeptide of any one of aspects 32-47.
- [00418] Aspect 49. The nucleic acid of aspect 48, wherein the nucleotide sequence is operably linked to a transcriptional control element.
- [00419] Aspect 50. The nucleic acid of aspect 49, wherein the transcriptional control element is a promoter.
- [00420] Aspect 51. The nucleic acid of aspect 50, wherein the promoter is a regulatable promoter.
- [00421] Aspect 52. The nucleic acid of aspect 50 or aspect 51, wherein the promoter is functional in a eukaryotic cell.
- [00422] Aspect 53. The nucleic acid of any one of aspects 48-52, further comprising a nucleotide sequence encoding a guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.
- [00423] Aspect 54. A recombinant expression vector comprising the nucleic acid of any one of aspects 48-53.
- [00424] Aspect 55. The recombinant expression vector of aspect 54, wherein the recombinant expression vector is recombinant viral expression vector.
- [00425] Aspect 56. A ribonucleoprotein (RNP) complex comprising:
- [00426] a) the fusion polypeptide of any one of aspects 32-47; and
- [00427] b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.
- [00428] Aspect 57. The RNP complex of aspect 56, wherein the guide RNA is a single-molecule guide RNA.
- [00429] Aspect 58. A composition comprising:
- [00430] a) the RNP complex of aspect 56 or aspect 57; and
- [00431] b) a pharmaceutically acceptable excipient.
- [00432] Aspect 59. The composition of aspect 58, comprising a lipid.

- [00433] Aspect 60. The composition of aspect 58, comprising a liposome, a hydrogel, a microparticle, a nanoparticle, or a block copolymer micelle.
- [00434] Aspect 61. A kit comprising:
- [00435] a) the RNA-guided effector polypeptide of any one of aspects 1-23, or the fusion polypeptide of any one of aspects 32-47; and
- [00436] b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.
- [00437] Aspect 62. The kit of aspect 61, wherein the guide RNA is a single-molecule guide RNA.
- [00438] Aspect 63. The kit of aspect 61 or aspect 62, wherein the RNA-guided effector polypeptide and the guide RNA are in separate containers.
- [00439] Aspect 64. The kit of aspect 61 or aspect 62, wherein the RNA-guided effector polypeptide and the guide RNA are together in a single container.
- [00440] Aspect 65. The kit of any one of aspects 61-64, wherein the guide RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, a Peptide Nucleic Acid, and a deoxyribonucleotide.
- [00441] Aspect 66. The kit of any one of aspects 61-65, further comprising a donor nucleic acid template.
- [00442] Aspect 67. A method of binding and/or modifying a target nucleic acid and/or modifying a polypeptide that binds to a target nucleic acid, the method comprising contacting the target nucleic acid with:
- [00443] i) the RNA-guided effector polypeptide of any one of aspects 1-23; or
- [00444] ii) the fusion polypeptide of any one of aspects 32-47.
- [00445] Aspect 68. The method of aspect 67, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA.
- [00446] Aspect 69. The method of aspect 67, wherein contacting results in genome editing.
- [00447] Aspect 70. The method of aspect 67 or 68, wherein said contacting takes place outside of a bacterial cell and outside of an archaeal cell.
- [00448] Aspect 71. The method of any one of aspects 67-69, wherein said contacting takes place *in vitro* outside of a cell.

- [00449] Aspect 72. The method of any one of aspects 67-69, wherein said contacting takes place inside of a target cell.
- [00450] Aspect 73. The method of aspect 72, wherein the target cell is a eukaryotic cell.
- [00451] Aspect 74. The method of aspect 72 or aspect 73, wherein the target cell is *in vivo*.
- [00452] Aspect 75. The method of aspect 72 or aspect 73, wherein the target cell is *ex vivo*.
- [00453] Aspect 76. The method of aspect 72 or aspect 73, wherein the eukaryotic cell is selected from the group consisting of: a plant cell, a fungal cell, a single cell eukaryotic organism, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell.
- [00454] Aspect 77. The method of any one of aspects 72-76, wherein said contacting further comprises: introducing a DNA donor template into the target cell.

EXAMPLES

- [00455] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

Example 1: Generation of deletion variants that retain target nucleic acid binding

MISER Protocol

- [00456] 1. A gene of interest is cloned into a suitable shuttle plasmid (e.g. pUC19).
- [00457] 2. Primers are designed such that they contain homology to 60 bp or more of the gene of interest, and a Type II restriction site (e.g. XbaI) is inserted into the middle of this primer. This process is then repeated for every single amino acid position of the protein such that, in the end, there are tiled primers capable of annealing at every amino acid position of the protein.

- [00458] 3. This process is repeated and a different, complementary restriction site (e.g. for XbaI, SpeI is suitable) is introduced into a second set of designed primers.
- [00459] 4. Primers can be synthesized via standard commercial synthesis. Olio Library Synthesis (OLS) primers (LeProust EM, et al. (2010) *Nucleic Acids Res* 38: 2522–2540; Kosuri et al. (2010) *Nat. Biotechnol.* 28:1295) were used; the OLS primers were obtained from Agilent.
- [00460] 5. Using recombineering (Higgins SA, Ouonkap SVY, Savage DF. 2017. Rapid and Programmable Protein Mutagenesis Using Plasmid Recombineering. *ACS Synth Biol* 6: 1825–1833.), these primers can be used to mutagenize the shuttle plasmid in a cell (e.g., in an *Escherichia coli* cell *in vitro*). This process systematically inserts the restriction site designed in 2 and 3, above, into the gene of interest once per gene but does so at every site of the gene across the population of plasmids.
- [00461] 6. This can be done in a variety of configurations but, briefly, in a typical work flow, recombineering would be carried out first for XbaI-containing primers, the plasmids would be minipreped, treated with XbaI to confirm they contain a single XbaI site, and then recombineering would be repeated a second time with SpeI. At the end of recombineering, the library is a diverse series of plasmids in which a single plasmid contains just one XbaI and one SpeI randomly distributed throughout the gene. All possible positions are represented across the library of plasmids,
- [00462] 7. The plasmid library is digested with XbaI and SpeI and plasmids are size-selected for electrophoresis or similar technique to select for desired deletion sizes. Recovered plasmids are re-ligated and transformed. (Cut XbaI and SpeI sites can ligated together).
- [00463] 8. This library now contains targeted deletions across the gene, where average deletion size is controllable by size-selection in step 7.
- [00464] 9. The protein library is assayed for function via a selection or screening assay. In the case of Cas9, one example of how this can be done is via a DNA-binding screen, e.g., as described in Oakes BL, Nadler DC, Savage DF. 2014. “Protein engineering of Cas9 for enhanced function.” *Methods in Enzymology* 546: 491–511. For example, catalytically inactive Cas9 (Cas9 lacking nuclease activity; “dCas9”) with a guide sequence of 5'-AACTTTCAGTTTAGCGGTCT-3' (SEQ ID NO:897) can target and repress a genome-encoded red fluorescent protein (RFP) while avoiding repression of a genome-encoded upstream green fluorescent protein (GFP). In a screening context, this provides a simple output for assaying dCas9 target binding functionality (i.e. RFP knock-down) while correcting for extrinsic noise in the population by monitoring GFP. Briefly, cells containing functional dCas9 (dCas9 that, when

complexed with a guide RNA, retains target nucleic acid binding) will repress RFP and express GFP while those with non-functional dCas9 will express both fluorescent proteins.

[00465] 10. Recovered libraries can be deep sequenced, the deletion site identified via bioinformatics, and compared to the initial library to quantitatively assess the functionality of a given deletion.

[00466] 11. This entire process gives all possible single contiguous deletions. Functional single deletions can be subjected to a second (or third, etc.) round to further investigate how deletions can be stacked together.

[00467] The above “MISER” protocol was used to generate deletions in Cas9 that retain the ability, when complexed with a guide RNA, to bind a target nucleic acid. Example data are shown in FIG. 8.

Example 2: Mammalian CRISPR interference assay to assess function of deletion variants

[00468] CRISPR interference (CRISPRi) assays (**FIGs. 24A-24E**) were used to assess the function of deletion variants in mammalian cells. Specifically, using a CRISPRi-based competitive proliferation assay, human U-251 glioblastoma cells were stably transduced with lentiviral vectors (pSC066) expressing MISER-dCas9 (MISER-DelA^{KRAB}, MISER-DelB^{KRAB}, MISER-DelC^{KRAB}, MISER-DelD^{KRAB}) or WT-dCas9 KRAB fusion proteins, followed by selection on puromycin. The positions of the DelA, DelB, DelC, and DelD deletions of the dCas9 polypeptide present in the dCas9-DelA^{KRAB}, dCas9-DelB^{KRAB}, dCas9-DelC^{KRAB}, and dCas9-DelD^{KRAB} fusions are as follows (amino acid numbering based on SEQ ID NO:5): delA: deletion of amino acids 180-297; delB: deletion of amino acids 503-708; delC: deletion of amino acids 792-897; and delD: deletion of amino acids 1010-1081. The respective cell lines were then transduced with a secondary lentiviral vector (pCF221) expressing mCherry fluorescent protein and either CRISPRi sgRNAs targeting essential genes proliferating cell nuclear antigen (PCNA) or replicating protein A1 (RPA1) (sgPCNA (sgRNAs i3, i4, and i6); or sgRPA1 (sgRNAs i1, i5, or i8) or non-targeting controls (sgNT (sgRNAs 1 and 2)). After mixing with the respective parental population (at approximately an 80:20 ratio of transduced to non-transduced cells), the percentage of mCherry positive cells was monitored by flow cytometry over several days to assess the effect of CRISPRi with the given Cas9-variant on cell proliferation. A functional CRISPRi variant is expected to inhibit essential gene expression, thus lowering the observed mCherry signal. Error bars indicate the standard deviation of triplicates. Significance in cell depletion was assessed by comparing samples to their respective day two controls using unpaired, two-tailed t-tests (alpha = 0.01).

[00469] FIG. 24A-24E depicts the percentage of mCherry positive mammalian cells in a CRISPR interference assay to assess the function in mammalian cells (human U-251 glioblastoma) of dCas9 variants with deletions. Figure 24A depicts the results using the WT (not deleted)-dCas9 construct fused to a KRAB repression domain. Sets of guide RNAs (CRISPRi sgRNAs) targeting three essential genes, proliferating cell nuclear antigen (PCNA) (sgPCNA: sgRNAs i3, i4, and i6) or replicating protein A1 (RPA1) (sgRPA1: sgRNAs i1, i5, or i8) or non-targeting controls (sgNT (sgRNAs 1 and 2)) where the target guide RNAs are also co-expressed with the mCherry fluorescent protein reporter. Cells were analyzed on days 2, 5, 9. The results demonstrate that the WT-cas9 KRAB construct is able to repress mCherry signal. Figure 24B depicts the same experiment using the MISER-DelA-KRAB fusion in place of the WT construct, and demonstrates that the amount of repression is less than with the WT cas9 construct while others demonstrated repression. The DelA construct comprises deletions in the dCas9 helix I and II regions. Figure 24C similarly depicts the results with the MISER-DelB-KRAB construct where none of the samples exhibited significant repression. The DelB construct comprises deletions in the dCas9 helix II regions. Figure 24D depicts the results with the MISER-DelC-KRAB construct and demonstrates that all of the samples demonstrated significant repression at day 9 as compared to day 2. The DelC construct comprises deletions in the dCas9 HNH nuclease domain. Figure 24E depicts the results with the MISER-DelD-KRAB construct and demonstrated that many of the samples show significant expression. The DelD construct comprises deletions in the dCas9 RuvC nuclease domain. (*) means significant repression compared to day 2 results of the grouping.

[00470] As shown in FIG. 24A, the wild-type (non-deleted) dCas9-KRAB fusion protein effectively inhibited PCNA expression and RPA1 expression. As shown in FIG. 24B, the DelA-dCas9-KRAB fusion protein (MISER-DelA^{KRAB}), combined with the i6 sgRNA, was effective in inhibiting PCNA gene expression. Similarly, as shown in FIG. 24B, the DelA-dCas9-KRAB fusion protein (MISER-DelA^{KRAB}), combined with the i5 sgRNA, was effective in inhibiting RPA1 gene expression. As shown in FIG. 24D, the DelC-dCas9-KRAB fusion protein (MISER-DelC^{KRAB}) (in combination with i3, i4, or i6 sgRNAs) effectively inhibited expression of PCNA; and (in combination with i1 or i5 sgRNAs) effectively inhibited expression of RPA1. As shown in FIG. 24E, the DelD-dCas9-KRAB fusion protein (MISER-DelD^{KRAB}) (in combination with i6 sgRNA) effectively inhibited expression of PCNA; and (in combination with i5 sgRNA) effectively inhibited expression of RPA1.

[00471] FIG. 25 provides an immunoblotting assay for Flag-tagged MISER-dCas9 or WT-dCas9 KRAB fusion proteins stably expressed in U-251 cells co-expressing a non-targeting guide (sgNT1). The indicated MISER deletions resulted in reduction of protein size. Beta-actin

(ACTB) was used as loading control. Protein ladders indicated reference molecular weight markers in kDa.

Example 3: *In vitro* binding of WT dCas9 and variants Δ 3CE, and Δ 4CE

[00472] *In vitro* function of WT-dCas9 and variants Δ 3CE and Δ 4CE was assessed using an RNP-based gel shift assay (**FIG. 26**). WT dCas9 and stacked variants Δ 3CE and Δ 4CE were expressed and purified to homogeneity from *E. coli*. Oligonucleotide-based target dsDNAs were designed possessing either a low (~30%, 298.2 tdTomato and DYRK1-BS14) or high (70%, B9) GC-content in the PAM proximal region. dsDNA was labeled on the 5' end of the non-target strand using either FAM or Cy5 fluorophores. RNP was formed following the protocol in Lin et al. (Lin et al. (2014) eLife; 3: e04766) with sgRNA against the respective target sequence. For gel shift, 300 nM RNP was incubated with 30 nM dsDNA target. The results were analyzed using gel electrophoresis and quantified using fluorescence of the gel-shifted fluorescently labeled dsDNA.

[00473] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An RNA-guided effector polypeptide having a length of from about 610 amino acids to about 1310 amino acids, wherein the RNA-guided effector polypeptide comprises one or more of:
 - i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;
 - ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
 - iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
 - d) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112,wherein the amino acid numbering is based on SEQ ID NO:5.
2. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324.
3. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739.
4. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.
5. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.
6. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324; and

ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739.

7. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324; and

ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.

8. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

i) a deletion of from 5 amino acids to 158 amino acids of amino acids 166-324; and

ii) a deletion of from 20 amino acids to 118 amino acids of amino acids 994-1112.

9. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;

ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and

iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.

10. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;

ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and

iii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.

11. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324;
- ii) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
- iii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- iv) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.

12. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and
- ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981.

13. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739; and
- ii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.

14. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of from 30 contiguous amino acids to 255 contiguous amino acids of amino acids 484-739;
- ii) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- iii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.

15. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of from 4 contiguous amino acids to 220 contiguous amino acids of amino acids 761-981; and
- ii) a deletion of from 20 contiguous amino acids to 118 contiguous amino acids of amino acids 994-1112.

16. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 179-296 based on the number of SEQ ID NO:5.

17. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 503-708 based on the number of SEQ ID NO:5.

18. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 792-897 based on the number of SEQ ID NO:5.

19. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.

20. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of amino acids 503-708 based on the number of SEQ ID NO:5;
- ii) a deletion of amino acids 792-897 based on the number of SEQ ID NO:5; and
- iii) a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.

21. The RNA-guided effector polypeptide of claim 1, wherein the RNA-guided effector polypeptide comprises:

- i) a deletion of amino acids 179-296 based on the number of SEQ ID NO:5;
- ii) a deletion of amino acids 503-708 based on the number of SEQ ID NO:5;
- iii) a deletion of amino acids 792-897 based on the number of SEQ ID NO:5; and
- iv) a deletion of amino acids 1010-1081 based on the number of SEQ ID NO:5.

22. The RNA-guided effector polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in FIG. 2, wherein the RNA-guided effector polypeptide is 990 amino acids in length.

23. The RNA-guided effector polypeptide of claim 1, wherein the polypeptide comprises an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence depicted in FIG. 3, wherein the RNA-guided effector polypeptide is 874 amino acids in length.

24. A nucleic acid comprising a nucleotide sequence encoding the RNA-guided effector polypeptide of any one of claims 1-23.

25. The nucleic acid of claim 24, wherein the nucleotide sequence is operably linked to a transcriptional control element.

26. The nucleic acid of claim 25, wherein the transcriptional control element is a promoter.

27. The nucleic acid of claim 26, wherein the promoter is a regulatable promoter.

28. The nucleic acid of claim 27, wherein the promoter is functional in a eukaryotic cell.

29. The nucleic acid of any one of claims 24-28, further comprising a nucleotide sequence encoding a guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.

30. A recombinant expression vector comprising the nucleic acid of any one of claims 24-29.

31. The recombinant expression vector of claim 30, wherein the recombinant expression vector is recombinant viral expression vector.

32. A fusion polypeptide comprising:

- a) the RNA-guided effector polypeptide of any one of claims 1-23; and
- b) a heterologous fusion partner.

33. The fusion polypeptide of claim 32, wherein the heterologous fusion partner provides for control of gene expression.

34. The fusion polypeptide of claim 32, wherein the heterologous fusion partner is a protein-modifying enzyme.

35. The fusion polypeptide of claim 32, wherein the heterologous fusion partner is a modifying enzyme is a DNA-modifying enzyme.

36. The fusion polypeptide of claim 32, wherein the heterologous fusion partner exhibits nuclease activity.

37. The fusion polypeptide of claim 36, wherein the nuclease activity is double-stranded DNA cleavage activity.

38. The fusion polypeptide of claim 37, wherein the heterologous fusion partner comprises an amino acid sequence having at least 85% amino acid sequence identity to the catalytic domain of the amino acid sequence depicted in FIG. 2A and has a length of no more than 200 amino acids.

39. The fusion polypeptide of any one of claims 32-38, wherein the fusion partner has a length of no more than 400 amino acids.

40. The fusion polypeptide of claim 35, wherein the heterologous fusion partner is a base editor.

41. The fusion polypeptide of claim 40, wherein the base editor is a cytidine deaminase.

42. The fusion polypeptide of claim 40, wherein the base editor is an adenosine deaminase.

43. The fusion polypeptide of claim 32, further comprising one or more nuclear localization sequences (NLS).

44. The fusion polypeptide of claim 43, wherein the fusion polypeptide comprises a single NLS.

45. The fusion polypeptide of claim 43, wherein the fusion polypeptide comprises 2 or more NLSs.

46. The fusion polypeptide of claim 43, wherein the fusion polypeptide comprises an NLS at the C-terminus, at the N-terminus, or at the C-terminus and the N-terminus, of the fusion polypeptide.

47. The fusion polypeptide of claim 32, further comprising an endosomolytic peptide.

48. A nucleic acid comprising a nucleotide sequence encoding the fusion polypeptide of any one of claims 32-47.

49. The nucleic acid of claim 48, wherein the nucleotide sequence is operably linked to a transcriptional control element.

50. The nucleic acid of claim 49, wherein the transcriptional control element is a promoter.

51. The nucleic acid of claim 50, wherein the promoter is a regulatable promoter.

52. The nucleic acid of claim 50 or claim 51, wherein the promoter is functional in a eukaryotic cell.

53. The nucleic acid of any one of claims 48-52, further comprising a nucleotide sequence encoding a guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.

54. A recombinant expression vector comprising the nucleic acid of any one of claims 48-53.

55. The recombinant expression vector of claim 54, wherein the recombinant expression vector is recombinant viral expression vector.

56. A ribonucleoprotein (RNP) complex comprising:

a) the fusion polypeptide of claim 32; and

b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.

57. The RNP complex of claim 56, wherein the guide RNA is a single-molecule guide RNA.
58. A composition comprising:
- a) the RNP complex of claim 56 or claim 57; and
 - b) a pharmaceutically acceptable excipient.
59. The composition of claim 58, comprising a lipid.
60. The composition of claim 58, comprising a liposome, a hydrogel, a microparticle, a nanoparticle, or a block copolymer micelle.
61. A kit comprising:
- a) the RNA-guided effector polypeptide of claim 1, or the fusion polypeptide of claim 32; and
 - b) a guide RNA, or a nucleic acid encoding the guide RNA, wherein the guide RNA comprises a guide sequence that is complementary to a target sequence of a target nucleic acid, and comprises a region that can bind to the RNA-guided effector polypeptide.
62. The kit of claim 61, wherein the guide RNA is a single-molecule guide RNA.
63. The kit of claim 61 or 62, wherein the RNA-guided effector polypeptide and the guide RNA are in separate containers.
64. The kit of claim 61 or 62, wherein the RNA-guided effector polypeptide and the guide RNA are together in a single container.
65. The kit of any one of claims 61-64, wherein the guide RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, a Peptide Nucleic Acid, and a deoxyribonucleotide.
66. The kit of any one of claims 61-65, further comprising a donor nucleic acid template.
67. A method of binding and/or modifying a target nucleic acid and/or modifying a polypeptide that binds to a target nucleic acid, the method comprising contacting the target nucleic acid with:
- i) the RNA-guided effector polypeptide of any one of claims 1-23; or

ii) the fusion polypeptide of any one of claims 32-47.

68. The method of claim 67, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA.

69. The method of claim 67, wherein contacting results in genome editing.

70. The method of claim 67 or 68, wherein said contacting takes place outside of a bacterial cell and outside of an archaeal cell.

71. The method of any one of claims 67-69, wherein said contacting takes place *in vitro* outside of a cell.

72. The method of any one of claims 67-69, wherein said contacting takes place inside of a target cell.

73. The method of claim 72, wherein the target cell is a eukaryotic cell.

74. The method of claim 72 or 73, wherein the target cell is *in vivo*.

75. The method of claim 72 or 73, wherein the target cell is *ex vivo*.

76. The method of claim 72 or 73, wherein the eukaryotic cell is selected from the group consisting of: a plant cell, a fungal cell, a single cell eukaryotic organism, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell.

77. The method of any one of claims 72-76, wherein said contacting further comprises: introducing a DNA donor template into the target cell.

FIG. 1

Spy Cas9

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDS
GETAEATRLKRTARRRYTRRKNRICYLQEIFS NEMAKVDDSFHRL EESFLVEE
DKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLR LIYLALAHMIKFR
GHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKILSARLSKSR
RLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLSKD TYDDDL
DNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQD
LTLLKALVRQQLP EKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGT
EELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIE
KILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERM
TNFDKNLPNEKVL PKHSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIV
DLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT YHDLKIIKDKD
FLDNEENEDILEDIVLT LTFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWG
RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG
QGDSLHEHIANLAGSPA IKKGILQTVKVDELVKVMGRHKPENIVIEMARENQTT
QKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMY
VDQELDINRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHV
AQILDSRMNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVREINNYHHAHD
AYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSN
IMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKK
TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEK
GKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLI IKLPKYSLFELN
GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIEQISEFSKR VILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNL
GAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD

(SEQ ID NO:5)

FIG. 3

D3CE:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDQYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLTSQKAQVSGQDSLHEHIANLAGSPAICK
GILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIKELASD
NLTKAERGGLSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVIT
LKSKLVSDFRKDFQFYKQVREINNYHHAHDAYLNAVVGITALIKKYPKLESEFVASTVRKV
LSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV
VAKVEKGKSKKLKSVKELLGITIMERSSEKPNIDFLEAKGYKEVKKDLIIKLPKYSLF
ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVEQHK
HYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH RD KPIREQAENI IHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD (SEQ ID
NO: 863)

FIG. 4

D4CE:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHAHMIKFRGHFLIEGDLNPD
NSTSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIP
HQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSE
ETITPWNFEVVVDKGASAQSFIERMTNFDKNLTSQKAQVSGQGDSLHEHIANLAGSPAI
KKGILQTVKVVDDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEDIKELA
SDNLTKAERGGELSELDKAGFIKQVLVETRQITKHVAQILDSRMNTKYDENDKLIREVKV
ITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGITALIKKYPKLESEFVASTVR
KVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSV
LVVAKVEKGKSKKLKSVKELLGITIMERSSEKPNIDFLEAKGYKEVKKDLIIKLPKYS
LFELNGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQ
HKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENI IHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD (SEQ ID
NO: 864)

FIG. 5A

spyCas9 (SEQ ID NO:865)

D3CE (SEQ ID NO:866)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE

MAKVDDSEFFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDS TDKADLRLLIYALAHMIKFRGHFLIEGDLNPDN
MAKVDDSEFFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDS TDKADLRLLIYALAHMIKFRGHFLIEGDLNPDN

SDVDKLFIQLVQTYNQLEENPINASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIASLSGLTPNFKSNFDLAEDAKLQLS
SDVDKLFIQLVQTYNQLEENPINASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIASLSGLTPNFKSNFDLAEDAKLQLS

KDYYDDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
KDYDDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK

NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILT
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILT

FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGM
FRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNL

RKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKKI IKDKDFLDNEENEDILEDIVLTL

TLFEDREMIERLKTYAHLFDDKVMQKLRRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQ
TSQKAQ

FIG. 5B

spyCas9 (SEQ ID NO:867)
D3CE (SEQ ID NO:868)

VSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIGELGSQLKEHPV
 VSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEEGIGEL

ENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVHDHIVPQSFCLKDDSIDNKKVLTTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK

LITQRFNDLTKAERGGLSELDKAGFIKRLQVETRQITKHVAQILD SRMNTKYDENDKLIREVKVI TLKSKLVSDFRKDFQFYKVREIN
 ASDNLTKAERGGLSELDKAGFIKRLQVETRQITKHVAQILD SRMNTKYDENDKLIREVKVI TLKSKLVSDFRKDFQFYKVREIN

NYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDKYVDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGE
 NYHHAHDAYLNAVVG TALIKKYPKLESEFV

TGEIVWDKGRDFATVRKVL SMPQVNI VKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKL
 ASTVRKVL SMPQVNI VKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKL

KSVKELLGITIMERSSEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFY LASHYEKLK
 KSVKELLGITIMERSSEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFY LASHYEKLK

GSPEDNEQQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
 GSPEDNEQQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR

YTSTKEVLDATLIHQSI TGLYETRIDLSQLGGD
 YTSTKEVLDATLIHQSI TGLYETRIDLSQLGGD

FIG. 6A

spyCas9 (SEQ ID NO: 898)
D4CE (SEQ ID NO: 899)
MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MDKKYSIGLAIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNE
MAKVDDSEFFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRLIYLALAHMIKFRGHFLIEGDLNPDN
MAKVDDSEFFHRLSEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRLIYLALAHMIKFRGHFLIEGDLNPDN
SDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS
KDYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
STSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSK
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFLLKDNREKIEKILT
NGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFLLKDNREKIEKILT
FRIPYVVGPLARGNSRFAWMTRKSEETITPWNFEEVVVDKGASQAQSFIERMTNFDKNLPNEKVLPHKSHLLYEYFTVYNELTKVKYVTEGM
FRIPYVVGPLARGNSRFAWMTRKSEETITPWNFEEVVVDKGASQAQSFIERMTNFDKNL
RKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTL
TLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQ
TSQKAQ
VSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTQKGQKNSRFRMKRIEIEGKELGSQILKEHPV
VSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKVMGRHKPENIVIAMARENQTQKGQKNSRFRMKRIEIEGKEL

FIG. 6B

spyCas9 (SEQ ID NO:900)
D4CE (SEQ ID NO:901)

ENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVHIVPQSFCLKDDSIDNKVLTNRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAK

LITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSPMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVVREIN
ASDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSPMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVVREIN

NYHHAHDAYLNAVVGITALIKKYPKLESEFVYGDYKVYDVRKMKIAKSEQEIGKATAKYFFYYSNIMNFFKTEITLANGEIRKRPLIETNGE
NYHHAHDAYLNAVVGITALIKKYPKLESEFV

TGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKL
ASTVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKL

KSVKELLGITIMERSSEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLIK
KSVKELLGITIMERSSEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLIK

GSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
GSPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR

YTSTKEVL DATLIHQSI TGLYETRIDLSQLGGD
YTSTKEVL DATLIHQSI TGLYETRIDLSQLGGD

FIG. 7A

FokI catalytic domain

QLVKSELEEKKSELRRHKLKYPHEYIELIEIARNSTQDRILEMKVMEFFMKVGYRGKHLGGSRKPDGAIYTVGSPIDYGVIVDTKAYS
GGYNLPIGQADEMQRYVEENQTRNKHINPNEWWKVYPSSVTEEFKFLFVSGHFKNYKAQLTRLNHI TNCNGAVLSVEELLIGGEMIKAG
TLTLEEVRRKFNNGEINF (SEQ ID NO: 869)

FIG. 7B

FokI cleavage domain Sharkey
GenBank ADI71347

196 aa

1 QLVKSELEEK KSELRRHKLKY VPHEYIELIE IARNPTQDRI LEMKVMEFFM KVGYRG~~EH~~L
61 GGSRKPDGAI YTVGSPIDYG VIVDTKAYSG GYNLPIGQAD EMQRYVEENQ TRNKHINPNE
121 WWKVYPSSVT EFKFLFVSGH FKGNKYKAQLT RLNHITNCNG AVL~~S~~VEELLI GGEMIKAGTL
181 TLEEVRRKFN NGEINF

(SEQ ID NO: 870)

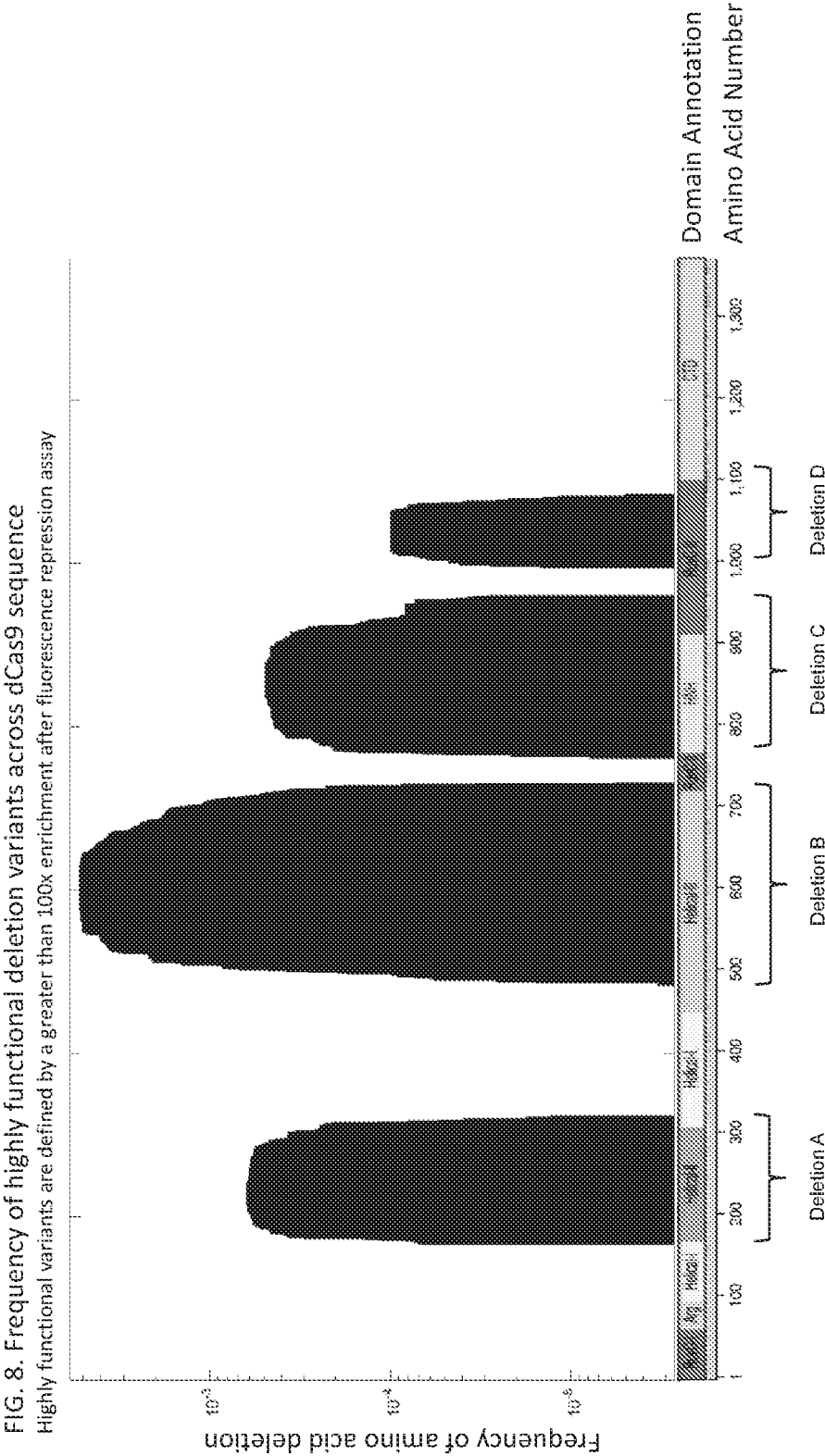


FIG. 9
Example minimal RNA-guided effector proteins

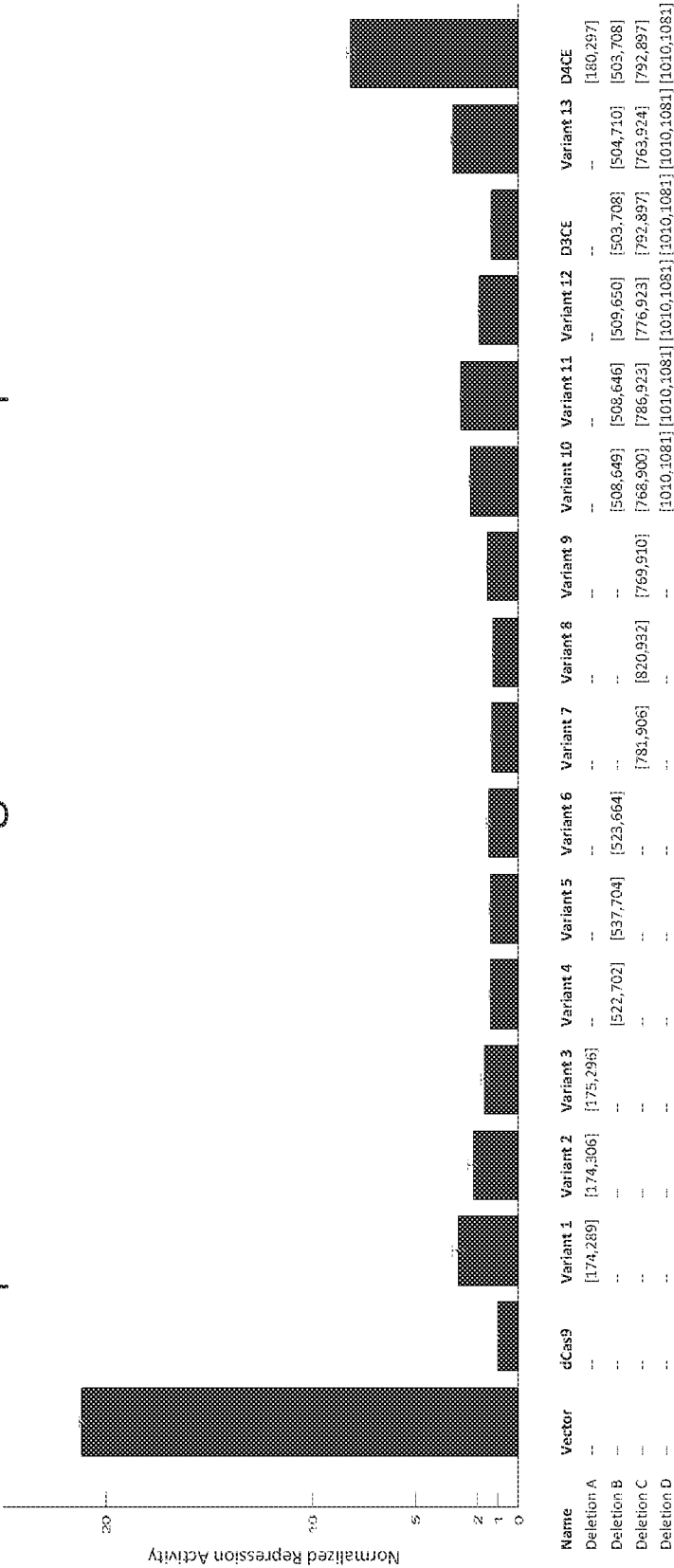


FIG. 10A

Variant 1 (Δ 174–289)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSEFFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLEENPINASCVDAKAILSARLSKSRLENLIAQLPCEKQNG
LFCNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQICDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIFILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDLVKVMGRHKPENIVIAMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGITALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPPKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEKKNPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFYLYLA
SHYEKLKGSPEDEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:871)

FIG. 10B

Variant 1 (Δ 174-289)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDFLAA
KNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQQLPEKYKEIFF
DQSKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIP
HQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMMTRKSE
ETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKY
VTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN
ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIEERLKTYAHLFDDKV
MKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI LDFLKS DGFANRNF MQLIHDDSLTFKE
DIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIEMAR
ENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
DQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKKNYW
RQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTK
YDENDKLIREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKY
PKLESEFVYGDYKVYDVRKMIKSEQEI GKATKYFFYSNIMNFFKTEITLANGEIRKR
PLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKL
IARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGSKKLKSVKELLGITIMERSSSFENP
IDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFY
LASHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQSI TGL
YETRIDLSQLGGD (SEQ ID NO:872)

FIG. 11A

Variant 2 (Δ 174-306)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLEENPINASCVDAKAILSARLSKSRLENLIAQLPCEKKNQ
~~LEGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQICDQYADLFLAKN~~
~~LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPPEKYKEIFFDQ~~
SKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLEFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:873)

FIG. 11B

Variant 2 (Δ 174-306)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDRVNT
EITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQ
EEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSI PHQIHLGELHAILRRQED
FYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGAS
AQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKK
AIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKD
FLDNEENEDILEDIVLTTLTFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSR
KLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEH
IANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMK
RIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHI
VPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
SKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDKVYDV
RKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDS
PTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSEKNPIDFLEAKGYKEVKKDLI
IKLPKYSLFELENGKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS PEDNEQ
KQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENI IHLF
TLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD
(SEQ ID NO:874)

FIG. 12A

Variant 3 (Δ 175-296)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLEENPINASCVDAKAILLSARLSKSRLENLIAQLPCEKKNQ
~~LEGNLIAISLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQICDQYADLFLAKN~~
~~LSDAILLS~~SDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLKIIKDKDFLDNEENEDILEDIVLTLTLEFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDLVKVMGRHKPENIVIAMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:875)

FIG. 12B

Variant 3 (Δ 175-296)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLSDA
ILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG
YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLG
ELHAILRRQEDFYFPFLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEETITPW
NFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMR
KPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT
HDLLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYAHLFDDKVMKQLKR
RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ
KGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI
NRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITLKSCLVSDFRKDFQFYKVBREINNYHHAHDAYLNAVVGTTALIKKYPKLESE
FVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETN
GETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD
WDPKKYGGFDSPTVAYSVLVVAKEGKSKKLKSVKELLGITIMERSSEKFNPIDFLEA
KGYKEVKKDLIIKLPKYSLFELNGRKRMLASAGELQKGNELALPSKYVNFLYLASHYE
KLKGSPEDNEQKQLFVEQHKHYLDEII EQISEFSKRVILADANLDKVL SAYNKH RDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRID
LSQLGGD (SEQ ID NO:876)

FIG. 13A

Variant 4 (Δ 522-702)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSLILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
~~EGRKPAFLSGEQKKAIVDLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS~~
~~LCTYHDLKLIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIEERLKYAHLEDDKVMK~~
~~QLKRRRYTCWCRLSRKLINGIRDKQSCKTILDFLKSDFANRNEMQLIHDDSLTFKEDI~~
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDLVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVHDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPPKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEKKNPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVI LADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:877)

FIG. 13B

Variant 4 (Δ 522-702)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHS IKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL EESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVDKLF IQLVQTYNQ LFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLS DILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLP EKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKF IKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFY PFLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVL PKHSLLEYFTVYTFKEDIQKAQ
VSGQGDSLHEHIANLAGSPA IKKGILQTVKVVDELVKVMGRHKPENIV IEMARENQTTQ
KGQKNSRERMKRIEEG IKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDI
NRLSDYD VDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQLLNA
KLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDK
LIREVKVITL KSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESE
FVYGDKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETN
GETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKD
WDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERS SFEKNPIDFLEA
KGYKEVKKDLIIKLPKYSLFEL ENGRKRMLASAGELQKGNE LALPSKYVNFLYLASHYE
KLKGSPEDNEQKQLFVEQHKHYLDEII EQISEFSKR VILADANLDKVLSAYNKHRDKPI
REQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYETRID
LSQLGGD (SEQ ID NO:878)

FIG. 14A
Variant 5 (Δ 537-704)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHS IKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL EESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVDKLFIQLVQTYNQLF EENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVD LLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRNAS
~~LCTYHDLLKLIKDKDFLDNEENEDILEDIVLTTLTTFEDREMIEERLKYAHLEDDKVMK~~
~~QLKRRRYTCWCRLSRKLINGIRDKQSCKTILDFLKSDGFANRNFMQLIHDDSLTEKEDI~~
QKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDL VKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKS KLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSF EKNPID
FLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNE LALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKR VILADANLDKVL SAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:879)

FIG. 14B

Variant 5 (Δ 537-704)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSLILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
EGMRKKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDDELVKVMGRHKPEN
IVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYLQ
NGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVV
KKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG
TALIKKYPKLESEFVYGDKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLA
NGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESILP
KRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMER
SSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPS
KYVNFLYLASHYEKLKGSPEDNEQQLFVEQHKHYLDEIIIEQISEFSKRVI LADANLDK
VLSAYNKHRRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLI
HQSITGLYETRIDLSQLGGD (SEQ ID NO:880)

FIG. 15A

Variant 6 (Δ 523-664)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEFVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
~~ECMRKPAFLSGEQKKAIVDLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS~~
~~LCTYHDLKIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKTVAHLEFDDKVMK~~
~~QLKRRRYTCWGRLSR~~KLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIAMAREN
QTTQKGQKNSRERMKRIEIEGIELGSQILKEHPVENTQLONEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETROITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPPKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNEALALPSKYVNFYLA
SHYEKLKGSPEQKQQLFVEQHKHYLDEIIIEQISEFSKRVLADANLKVLSAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSIITGLYE
TRIDLSQLGGD (SEQ ID NO:881)

FIG. 15B

Variant 6 (Δ 523-664)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDDSFFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNKLINGIRDK
QSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKEL
GSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKMKKNYWRQLLNAKLITQRKFDNLTKAERGGLS
ELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRK
DFQFYKVREINNYHHAHDAYLNAVVGITALIKKYPKLESEFVYGDKVYDVRKMIKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKV
LSMPQVNIVKKTEVQTTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV
VAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDIIKLPKYSLF
ELENKRKRLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEQNEQQLFVEQHK
HYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH RD KPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD (SEQ ID
NO:882)

FIG. 16A
Variant 7 (Δ 781-906)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKN
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMOLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIIEGCIKELGSOILKEHPVENTQLONEKLYLYYLQNGRDMYVDO
~~ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTSDKNRCKSDNVPSEEVVKMKKNYWRQ~~
~~LLNAKLITQRKFDNLTKAERGGLSEL~~DKAGFIKRQLVETRQITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKPREINNYHHAHDAYLNAVVGITALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEKKNPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGSPEQKQQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:883)

FIG. 16B
Variant 7 (Δ 781-906)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIEERLKYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERGLSELDKAGFIKQQLVETRQITKHVAQILDSRMNTKYDENDKLIRE
VKVITLKSCLVSDFRKDFQFYKQVREINNYHHAHDAYLNAVVGITALIKKYPKLESEFVYG
DYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
EIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKELLGITIMERSSEKNPIDFLEAKGYK
EVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKHDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQSI TGLYETRIDLSQL
GGD (SEQ ID NO:884)

FIG. 17A

Variant 8 (Δ 820-932)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSEFFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNQ
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTQVQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIIEGikelGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDO
~~ELDINRLSDYDVDHIVPOSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKKNYWRQ~~
~~LLNAKLITQRKFDNLTKAERCCLSELDKACFIKRQLVETROITKHVAQILDSRMNTKYD~~
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:885)

FIG. 17B

Variant 8 (Δ 820-932)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSEFFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGL
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEET
ITPWNFEDEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIVIEMAREN
QTTQKGQKNSRERMKRIIEGIELGSQILKEHPVENTQLQNEKLYLYYLQNGQILDSRM
NTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAII
KKYPKLESEFVYGDYKVYDVRKMIKSEQEI GKATKYFFYSNIMNFFKTEITLANGEI
RKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSE
KNPIDFLEAKGYKEVKKDLIIKLPKYSLFELNGRKRMLASAGELQKGNELALPSKYVN
FLYLASHYEKLKGSPEQKQLFVEQHKHYLDEIIIEQISEFSKRVI LADANLDKVLSA
YNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQSI
TGLYETRIDLSQLGGD (SEQ ID NO:886)

FIG. 18A
Variant 9 (Δ 769-910)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHS IKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIFKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGTYHDLLKI IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDDELVKVMGRHKPENIVIEMAREN
QTTQKCKNSRERPMKRIFEGIKELGSQLKEHPVENTQLONEKLYLYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRCKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERGCLSELDKAGFIKRQLVETROITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLFELENGKRMLASAGELQKGNEALALPSKYVNFLYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVI LADANLDKVL SAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:887)

FIG. 18B

Variant 9 (Δ 769-910)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSLILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIFKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
EGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS
LGT YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMK
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF MQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIVIAMAREN
QLDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRK
DFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVYGDYKVYDVRKMIKSEQ
EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKV
LSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLV
VAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKKDLIIKLPKYSLF
ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLLKGSPEDNEQKQLFVEQHK
HYLDEIIEQISEFSKRVLADANLDKVL SAYNKH RD KPIREQAENIIHLFTLTNLGAPA
AFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD (SEQ ID
NO:888)

FIG. 19A

Variant 10 (Δ 508-649; Δ 768-900; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKDLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVVGPLARGNSRFAWMTRKSEET
ITPWNFEFVVDKGASAQSFIERMTNFDKNLPNEKVL~~LPKHSLLYEYFTVYNELTKVKYVT~~
~~EGMRKPAFLSGEQKKAIVDLEKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS~~
~~LCTYHDLLKIIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIEERLKTVAHLEDDKVMK~~
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDLVKVMGRHKPENIVIEMAREN
~~QTTQKGCQKNSRERPMKRITTEGKELGSQLKEHPVENTQLONEKLYLYLQNGRDMYVDQ~~
~~ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRCKSDNVPSEEVVKKMKNYWRQ~~
~~LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETROITKHVAQILDSRMNTKYD~~
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKAKSEGEIGKATAKYFFYSNIMNFEKTEITLANGEIRKRL
~~IETNGETCEIVWDKGRDFA~~TVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA
RKKDWDPPKYGGFDSPTVAYSVLVAVKEGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFYLA
SHYEKLKGSPEDEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:889)

FIG. 19B

Variant 10 (Δ 508-649; Δ 768-900; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSEFFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNQ
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYYVGPLARGNSRFAWMTRKSEET
ITPWNFEVVDKGGASQSFIERMTNFDKNLPNEKVQLKRRRYTGWGRLSRKLINGIRDK
QSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDDELVKVMGRHKPENIVIAMARENTKAERGGLSELDKAGFIKRQLVET
RQITKHVAQILD SRMNTKYDENDKLIREVKVITLKS KLVSDFRKDFQFYKVREINNYHH
AHDAYLNAVVG TALIKKYPKLESEFVTVRKVLSMPQVNIVKKTEVQTGGFSKESILPKR
NSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKELLGITIMERS
FEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKY
VNFLYLASHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL
SAYNKH RD KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGD (SEQ ID NO:890)

FIG. 20A

Variant 11 (Δ 508-646; Δ 786-923; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLV DSTKADLR LIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAMWTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVL~~PKHSLLYEYFTVYNELTKVKYVT~~
~~ECMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS~~
~~LCTYHDLKLIKDKDFLDNEENEDILEDIVLTTLTFEDREMIERLKTVAHLEDDKVMK~~
QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFMQLIHDDSLTFKEDI
QKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIVIAMAREN
QTTQKGQKNSRERMKRIIEGKELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQ
~~ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQ~~
~~LLNAKLITQRKFDNLTKAERCGLSELDKACFIKRQLVETRQITKHVAQILDSRMNTKYD~~
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEGEICKATAKYFEYSNIMNFEKTEITLANCEIRKRL
~~IETNGETCEIIVWDKGRDFATVRKVL SMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA~~
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFENPID
FLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLA
SHYEKLKGSPEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANL DKVLSAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:891)

FIG. 20B

Variant 11 (Δ 508-646; Δ 786-923; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSLILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIFILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVVMKQLKRRRYTGWGRLSRKLINGI
RDKQSGKTILDFLKSDGFANRNFMLIHDDSLTFKEDIQKAQVSGQGDLSLHEHIANLAG
SPAIKKGILQTVKVVDLKVVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIETRQ
ITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAH
DAYLNAVVGITALIKKYPKLESEFVTVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNS
DKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEFE
KNPIDFLEAKGYKEVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVN
FLYLASHYEKLKGSPEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVLISA
YNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSI
TGLYETRIDLSQLGGD (SEQ ID NO:892)

FIG. 21A

Variant 12 (Δ 509-650; Δ 776-923; Δ 1010-1081)

MDKKYSIGLDIGTNSVGVAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEET
ITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
~~ECMRKPAFLSCEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNAS~~
~~LCTYHDLKLIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYAHLEFDDKVMK~~
~~QLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDI~~
QKAQVSGQDSLHEHIANLAGSPAIIKKGILQTVKVVDDELVKVMGRHKPENIVIAMAREN
QTTQKGQKNSRERMKRIIEGKELGSGQILKEHPVENTQLONEKLYLYLQNGRDMYVDO
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRCKSDNVPSEEVVKKMKNYWRQ
LLNAKLITQRKFDNLTKAERCGLSELDKACFIKRQLVETROITKHVAQILDSRMNTKYD
ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVG TALIKKYPK
LESEFVYGDYKVYDVRKMIKSEGEICKATAKYFFYSNIMNFEKTEITLANCEIRKRPL
~~IETNGETCEIIVWDKGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA~~
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSEFEKNPID
FLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLA
SHYEKLKGS PEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKHR
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:893)

FIG. 21B

Variant 12 (Δ 509-650; Δ 776-923; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKN
LFGNLIALLSLGLTPNFKSNFDLAEDAKLQLSKDYYDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLLKRRRYTGWGRLSRKLINGIRDK
QSGKTILDFLKSDGFANRNFQMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPA
IKKGILQTVKVVDDELVKVMGRHKPENIVIVEMARENQTTQKGQKTRQITKHVAQILDSRM
NTKYDENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTA
KKYPKLESEFVTVRKVLSPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSPTVAYSVLVVAKEKGKSKKLKSVKELLGITIMERSSEKKNPIDFLEAKGYK
EVKKDLIIKLPKYSLELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKH
RDKPIREQA
ENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLIHQSI
TGLYETRIDLSQL
GGD (SEQ ID NO:894)

FIG. 22A

Variant 13 (Δ 504-710; Δ 763-924; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALSGLTPNFKSNFDLAEDAKLQLSKDYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPKEYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVT
~~ECMRKPAFLSCEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISCVEDRFNAS~~
~~LCTYHDLLKLIKDKDFLDNEENEDILEDIVLTTLTLFEDREMIERLKYAHLEFDDKVMK~~
~~QLKRRRYTCWCRLSRKLINCIRDKQSCKTILDFLKSDGFANRNEMQLIHDDSLTFKEDI~~
~~QKAQVSGQDSLHEHIANLAGSPAIKKGILQTVKVVDDELVKVMGRHKPENIVIAMAREN~~
~~OTTQKQKNSRERMKRIIEGKELGSGQILKEHPVENTQLONEKLYLYLQNGRDMYVDQ~~
~~ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRCKSDNVPSEEVVKKMKNYWRQ~~
~~LLNAKLITQRKFDNLTKAERCGLSELDKACFIKRQLVETRQITKHVAQILDSRMNTKYD~~
~~ENDKLIREVKVITLKSCLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTAIIKKYPK~~
~~LESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFEKTEITLANCEIRKRL~~
~~IETNGETCEIIVWDKGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIA~~
RKKDWDPKKYGGFDSPTVAYSVLVAKVEKGKSKKLKSVKELLGITIMERSSSFKNPID
FLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFYLA
SHYEKLKGSPEQKQLFVEQHKHYLDEIIIEQISEFSKRVLADANLDKVL SAYNKH
DKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQ SITGLYE
TRIDLSQLGGD (SEQ ID NO:895)

FIG. 22B

Variant 13 (Δ 504-710; Δ 763-924; Δ 1010-1081)

MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETA
EATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPI
FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLLIYLALAHMIKFRGHFLIEGDLNPD
NSDVKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNG
LFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKD TYDDDLNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQ
SKNGYAGYIDGGASQEEFYKFIPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQ
IHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEET
ITPWNFEEVVDKGASAQSFIERMTNFDKNLPAQVSGQDSLHEHIANLAGSPAIKKGIL
QTVKVVDDELVKVMGRHKPENIVIERQITKHVAQILDSRMNTKYDENDKLIREVKVITLK
SKLVSDFRKDFQFYKVBREINNYHHAHDAYLNAVVG TALIKKYPKLESEFVTVRKVLSMP
QVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKV
EKGKSKKLKSVKELLGITIMERSSEKKNPIDFLEAKGYKEVKKDLIIKL PKYSLFELEN
GRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGS PEDNEQKQLFVEQHKHYLD
EIIEQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHLFTLTNLGAPAAFKY
FDTTIDRKRYTSTKEVL DATLIHQ SITGLYETRIDLSQLGGD (SEQ ID NO:896)

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
166	176	484	637	761	785	994	1037
166	178	484	638	761	826	994	1039
166	185	484	649	761	835	994	1043
166	191	484	660	761	856	994	1044
166	194	484	661	761	901	994	1048
166	210	484	677	761	914	994	1051
166	214	484	680	761	917	994	1053
166	227	484	687	761	955	994	1056
166	228	484	700	762	797	994	1058
166	229	485	535	762	811	994	1062
166	233	485	626	762	834	994	1063
166	237	485	644	762	848	994	1064
166	243	485	646	762	852	994	1065
166	245	485	650	762	855	994	1066
166	250	485	677	762	856	994	1067
166	261	485	688	762	857	994	1068
166	272	485	725	762	864	994	1069
166	273	486	545	762	877	994	1070
166	275	486	562	762	879	994	1071
166	276	486	627	762	881	994	1072
166	286	486	635	762	889	994	1073
166	287	486	643	762	890	994	1074

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
166	291	486	647	762	895	994	1075
166	294	486	652	762	898	994	1079
166	305	486	654	762	900	994	1083
166	306	486	671	762	901	994	1086
166	308	486	672	762	904	994	1090
166	311	486	673	762	909	994	1094
166	314	486	678	762	910	994	1096
166	316	486	682	762	916	994	1104
167	171	486	687	762	920	994	1105
167	173	486	715	762	924	994	1106
167	175	486	730	762	925	994	1110
167	176	486	736	762	928	995	1014
167	177	486	739	763	769	995	1017
167	178	487	524	763	771	995	1032
167	179	487	529	763	779	995	1035
167	181	487	539	763	785	995	1037
167	182	487	604	763	789	995	1039
167	185	487	605	763	793	995	1042
167	187	487	618	763	810	995	1044
167	189	487	635	763	814	995	1045
167	191	487	636	763	823	995	1046
167	193	487	637	763	824	995	1048

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
167	194	487	640	763	826	995	1051
167	195	487	645	763	829	995	1056
167	198	487	646	763	835	995	1057
167	199	487	647	763	847	995	1058
167	200	487	648	763	850	995	1062
167	201	487	651	763	851	995	1063
167	217	487	653	763	852	995	1064
167	220	487	661	763	853	995	1065
167	222	487	673	763	854	995	1066
167	223	487	678	763	856	995	1067
167	224	487	679	763	857	995	1068
167	226	487	685	763	858	995	1069
167	227	487	686	763	859	995	1070
167	228	487	688	763	860	995	1071
167	229	487	690	763	867	995	1072
167	232	487	698	763	870	995	1073
167	233	487	701	763	880	995	1074
167	234	487	705	763	881	995	1075
167	235	487	710	763	882	995	1076
167	237	487	713	763	892	995	1077
167	239	487	716	763	893	995	1079
167	242	487	724	763	895	995	1081

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
167	243	487	725	763	897	995	1091
167	244	488	536	763	899	995	1094
167	245	488	545	763	902	995	1095
167	246	488	623	763	904	995	1108
167	247	488	641	763	905	995	1111
167	248	488	645	763	906	996	1026
167	249	488	646	763	910	996	1044
167	250	488	649	763	912	996	1049
167	253	488	652	763	916	996	1056
167	254	488	654	763	917	996	1058
167	255	488	660	763	920	996	1062
167	256	488	672	763	921	996	1066
167	257	488	674	763	924	996	1067
167	259	488	686	763	934	996	1075
167	260	488	703	763	937	996	1076
167	261	488	709	763	978	997	1019
167	263	488	720	764	768	997	1023
167	264	488	728	764	769	997	1024
167	265	489	572	764	777	997	1026
167	268	489	638	764	785	997	1028
167	271	489	643	764	798	997	1029
167	272	489	645	764	813	997	1030

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
167	273	489	646	764	823	997	1031
167	274	489	647	764	827	997	1032
167	275	489	649	764	832	997	1036
167	276	489	653	764	835	997	1037
167	277	489	654	764	836	997	1038
167	278	489	655	764	837	997	1039
167	279	489	658	764	841	997	1040
167	280	489	660	764	842	997	1041
167	281	489	661	764	850	997	1042
167	283	489	662	764	852	997	1043
167	284	489	665	764	853	997	1044
167	285	489	682	764	854	997	1045
167	287	489	687	764	855	997	1046
167	289	489	692	764	856	997	1047
167	290	489	698	764	857	997	1048
167	291	489	703	764	858	997	1049
167	292	489	704	764	861	997	1051
167	294	489	708	764	873	997	1053
167	295	489	731	764	877	997	1054
167	296	490	521	764	878	997	1055
167	297	490	545	764	879	997	1056
167	300	490	573	764	880	997	1057

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
167	301	490	597	764	881	997	1058
167	304	490	609	764	882	997	1059
167	305	490	643	764	883	997	1061
167	306	490	644	764	885	997	1062
167	307	490	645	764	886	997	1064
167	309	490	646	764	891	997	1065
167	311	490	647	764	892	997	1066
167	312	490	648	764	893	997	1067
167	313	490	649	764	894	997	1068
167	314	490	650	764	895	997	1069
167	315	490	651	764	899	997	1070
167	316	490	652	764	902	997	1071
167	317	490	653	764	903	997	1072
168	173	490	654	764	904	997	1073
168	179	490	658	764	905	997	1074
168	180	490	659	764	906	997	1075
168	184	490	660	764	907	997	1076
168	193	490	661	764	908	997	1077
168	222	490	664	764	909	997	1078
168	228	490	669	764	910	997	1079
168	233	490	672	764	912	997	1080
168	238	490	674	764	915	997	1081

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
168	242	490	686	764	916	997	1083
168	243	490	687	764	918	997	1089
168	244	490	688	764	919	997	1090
168	246	490	698	764	920	997	1098
168	250	490	702	764	921	997	1100
168	253	490	707	764	922	997	1101
168	255	490	708	764	923	997	1105
168	257	490	709	764	924	997	1110
168	260	490	716	764	925	998	1021
168	261	490	724	764	927	998	1022
168	264	490	725	764	929	998	1023
168	265	490	729	764	932	998	1025
168	271	490	730	764	935	998	1029
168	272	490	736	764	937	998	1030
168	276	491	521	764	938	998	1032
168	279	491	647	764	943	998	1035
168	284	491	653	764	944	998	1036
168	287	491	654	764	958	998	1037
168	294	491	658	764	959	998	1038
168	295	491	660	764	960	998	1039
168	297	491	661	765	769	998	1040
168	299	491	672	765	776	998	1043

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
168	300	491	729	765	798	998	1044
168	301	492	572	765	810	998	1045
168	305	492	645	765	814	998	1046
168	306	492	647	765	826	998	1047
168	312	492	648	765	848	998	1048
168	316	492	650	765	849	998	1050
168	317	492	653	765	855	998	1051
168	318	492	654	765	858	998	1053
169	173	492	655	765	859	998	1056
169	194	492	660	765	863	998	1057
169	199	492	663	765	869	998	1058
169	201	492	666	765	871	998	1059
169	216	492	673	765	880	998	1061
169	222	492	675	765	890	998	1062
169	228	492	685	765	892	998	1064
169	230	492	686	765	894	998	1065
169	235	492	687	765	898	998	1066
169	243	492	704	765	904	998	1067
169	244	492	709	765	909	998	1068
169	246	493	635	765	915	998	1069
169	250	493	641	765	916	998	1070
169	253	493	643	765	917	998	1071

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
169	255	493	644	765	918	998	1072
169	263	493	645	765	919	998	1073
169	264	493	646	765	920	998	1074
169	271	493	647	765	921	998	1075
169	273	493	649	765	922	998	1076
169	278	493	650	765	923	998	1077
169	279	493	652	765	924	998	1078
169	283	493	654	765	925	998	1080
169	287	493	656	765	956	998	1081
169	289	493	659	766	773	998	1083
169	291	493	661	766	774	998	1095
169	294	493	662	766	776	998	1096
169	295	493	663	766	777	998	1098
169	296	493	665	766	785	998	1100
169	297	493	673	766	786	998	1101
169	301	493	674	766	791	998	1108
169	302	493	675	766	795	998	1110
169	305	493	680	766	799	998	1112
169	306	493	683	766	806	999	1029
169	312	493	684	766	811	999	1037
169	314	493	685	766	815	999	1038
169	316	493	687	766	817	999	1042

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
169	317	493	688	766	822	999	1044
169	318	493	692	766	824	999	1046
169	321	493	697	766	827	999	1047
170	178	493	702	766	828	999	1058
170	191	493	704	766	832	999	1067
170	193	493	710	766	833	999	1068
170	200	493	711	766	835	999	1069
170	205	494	529	766	836	999	1070
170	216	494	544	766	841	999	1071
170	218	494	573	766	842	999	1072
170	226	494	621	766	843	999	1073
170	228	494	625	766	844	999	1074
170	229	494	626	766	847	999	1075
170	242	494	641	766	850	999	1079
170	243	494	642	766	851	999	1095
170	244	494	643	766	852	999	1105
170	246	494	644	766	854	999	1110
170	250	494	645	766	856	1000	1023
170	252	494	646	766	857	1000	1032
170	253	494	647	766	860	1000	1037
170	254	494	649	766	865	1000	1043
170	255	494	652	766	866	1000	1061

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
170	259	494	653	766	867	1000	1062
170	260	494	654	766	868	1000	1066
170	271	494	656	766	871	1000	1067
170	272	494	658	766	877	1000	1068
170	275	494	659	766	879	1000	1069
170	287	494	660	766	881	1000	1073
170	289	494	661	766	882	1000	1074
170	293	494	664	766	883	1000	1075
170	295	494	665	766	884	1000	1076
170	297	494	671	766	885	1000	1081
170	302	494	672	766	886	1000	1106
170	306	494	673	766	890	1001	1021
170	308	494	674	766	891	1001	1029
170	312	494	675	766	892	1001	1038
170	314	494	680	766	893	1001	1039
170	315	494	682	766	895	1001	1043
170	318	494	684	766	897	1001	1046
170	319	494	685	766	898	1001	1047
171	176	494	686	766	899	1001	1048
171	206	494	687	766	901	1001	1049
171	214	494	688	766	902	1001	1051
171	216	494	697	766	903	1001	1053

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
171	228	494	698	766	904	1001	1056
171	233	494	699	766	906	1001	1058
171	240	494	700	766	907	1001	1059
171	242	494	701	766	908	1001	1061
171	243	494	702	766	910	1001	1062
171	244	494	703	766	912	1001	1065
171	246	494	704	766	915	1001	1066
171	247	494	705	766	916	1001	1067
171	248	494	706	766	919	1001	1068
171	249	494	708	766	920	1001	1069
171	250	494	709	766	921	1001	1070
171	253	494	710	766	922	1001	1071
171	255	494	711	766	923	1001	1072
171	260	494	712	766	924	1001	1073
171	261	494	713	766	925	1001	1074
171	263	494	716	766	926	1001	1075
171	272	495	635	766	928	1001	1076
171	274	495	660	766	929	1001	1077
171	281	495	673	766	932	1001	1078
171	284	496	563	766	934	1001	1079
171	290	496	606	766	950	1001	1080
171	292	496	640	766	951	1001	1081

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
171	294	496	645	766	953	1001	1082
171	296	496	646	766	957	1001	1083
171	297	496	647	766	958	1001	1085
171	302	496	650	766	959	1001	1089
171	305	496	655	766	960	1001	1093
171	306	496	658	766	961	1001	1106
171	313	496	660	766	981	1001	1108
171	314	496	661	767	776	1001	1110
171	316	496	664	767	780	1001	1111
171	318	496	673	767	785	1002	1023
171	321	496	675	767	797	1002	1029
172	179	496	688	767	798	1002	1032
172	184	496	702	767	810	1002	1033
172	185	496	705	767	811	1002	1037
172	186	496	712	767	817	1002	1038
172	190	496	723	767	824	1002	1039
172	197	496	724	767	825	1002	1040
172	201	497	563	767	826	1002	1043
172	203	497	645	767	829	1002	1046
172	211	497	646	767	832	1002	1047
172	212	497	649	767	834	1002	1053
172	213	497	654	767	835	1002	1057

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
172	214	497	658	767	837	1002	1058
172	216	497	659	767	843	1002	1059
172	217	497	660	767	845	1002	1061
172	222	497	661	767	848	1002	1062
172	224	497	670	767	850	1002	1063
172	228	497	673	767	855	1002	1065
172	229	497	683	767	856	1002	1066
172	230	497	684	767	857	1002	1067
172	231	497	688	767	858	1002	1068
172	238	497	690	767	859	1002	1069
172	240	497	693	767	867	1002	1070
172	242	497	705	767	868	1002	1071
172	243	497	712	767	869	1002	1072
172	244	497	716	767	871	1002	1073
172	246	497	725	767	873	1002	1074
172	247	498	536	767	878	1002	1075
172	250	498	544	767	880	1002	1076
172	251	498	548	767	881	1002	1077
172	253	498	552	767	882	1002	1078
172	254	498	559	767	885	1002	1079
172	255	498	562	767	892	1002	1080
172	257	498	580	767	897	1002	1081

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
172	260	498	585	767	898	1002	1083
172	261	498	598	767	899	1002	1100
172	265	498	600	767	901	1002	1103
172	271	498	601	767	902	1002	1104
172	272	498	602	767	904	1003	1025
172	273	498	603	767	906	1003	1029
172	274	498	609	767	910	1003	1036
172	275	498	621	767	911	1003	1037
172	276	498	626	767	913	1003	1038
172	278	498	628	767	915	1003	1039
172	279	498	630	767	916	1003	1043
172	284	498	631	767	917	1003	1045
172	285	498	635	767	919	1003	1047
172	286	498	636	767	920	1003	1051
172	287	498	638	767	921	1003	1053
172	288	498	640	767	922	1003	1056
172	289	498	641	767	923	1003	1057
172	294	498	642	767	924	1003	1058
172	295	498	643	767	925	1003	1059
172	297	498	644	767	927	1003	1061
172	301	498	645	767	928	1003	1062
172	302	498	646	767	929	1003	1065

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
172	304	498	647	767	931	1003	1066
172	305	498	648	767	932	1003	1067
172	306	498	649	767	933	1003	1068
172	311	498	650	767	937	1003	1069
172	312	498	651	767	939	1003	1070
172	314	498	652	767	948	1003	1071
172	316	498	653	767	949	1003	1072
172	317	498	654	767	955	1003	1073
172	318	498	656	767	956	1003	1074
172	319	498	657	767	957	1003	1075
172	320	498	658	767	959	1003	1076
172	321	498	659	767	960	1003	1077
172	322	498	660	767	961	1003	1078
172	324	498	661	767	968	1003	1079
173	179	498	662	767	971	1003	1080
173	185	498	663	768	782	1003	1081
173	189	498	664	768	789	1003	1082
173	193	498	665	768	798	1003	1083
173	194	498	667	768	804	1003	1084
173	195	498	668	768	810	1003	1087
173	196	498	669	768	813	1003	1093
173	197	498	670	768	825	1003	1094

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
173	199	498	671	768	826	1003	1096
173	203	498	672	768	827	1003	1101
173	210	498	673	768	832	1003	1104
173	216	498	674	768	835	1003	1108
173	218	498	675	768	836	1003	1110
173	220	498	676	768	846	1003	1112
173	222	498	677	768	848	1004	1023
173	223	498	678	768	849	1004	1026
173	226	498	679	768	851	1004	1029
173	228	498	680	768	853	1004	1032
173	231	498	681	768	854	1004	1037
173	234	498	682	768	856	1004	1038
173	235	498	683	768	857	1004	1039
173	242	498	684	768	858	1004	1042
173	243	498	685	768	866	1004	1043
173	244	498	686	768	870	1004	1044
173	245	498	687	768	880	1004	1045
173	246	498	688	768	881	1004	1046
173	247	498	689	768	882	1004	1047
173	249	498	690	768	883	1004	1049
173	250	498	692	768	884	1004	1056
173	251	498	693	768	885	1004	1057

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
173	253	498	696	768	889	1004	1058
173	255	498	697	768	892	1004	1059
173	257	498	698	768	893	1004	1060
173	261	498	699	768	897	1004	1061
173	265	498	700	768	899	1004	1062
173	266	498	701	768	900	1004	1063
173	271	498	702	768	901	1004	1064
173	272	498	703	768	902	1004	1065
173	273	498	704	768	903	1004	1066
173	274	498	705	768	904	1004	1067
173	275	498	706	768	906	1004	1068
173	276	498	708	768	908	1004	1069
173	278	498	709	768	910	1004	1070
173	281	498	710	768	912	1004	1071
173	287	498	711	768	915	1004	1072
173	288	498	712	768	916	1004	1073
173	289	498	713	768	917	1004	1074
173	290	498	714	768	919	1004	1075
173	291	498	715	768	920	1004	1076
173	294	498	716	768	921	1004	1077
173	295	498	717	768	922	1004	1078
173	296	498	720	768	923	1004	1079

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
173	297	498	721	768	924	1004	1080
173	300	498	722	768	925	1004	1081
173	301	498	723	768	929	1004	1083
173	302	498	724	768	932	1004	1084
173	305	498	725	768	933	1004	1085
173	306	498	726	768	935	1004	1093
173	307	498	729	768	937	1004	1094
173	311	499	537	768	947	1004	1101
173	312	499	548	768	950	1004	1103
173	314	499	562	768	951	1004	1106
173	316	499	568	768	954	1005	1024
173	317	499	571	768	959	1005	1026
173	318	499	573	768	960	1005	1028
173	319	499	589	768	961	1005	1029
173	323	499	602	768	964	1005	1030
174	178	499	611	768	968	1005	1031
174	179	499	620	769	774	1005	1032
174	181	499	621	769	776	1005	1036
174	183	499	626	769	785	1005	1037
174	184	499	628	769	786	1005	1040
174	185	499	631	769	795	1005	1041
174	187	499	632	769	796	1005	1042

FIG. 23

FIG. 23

Deletion A		Deletion B		Deletion C		Deletion D	
Start	End	Start	End	Start	End	Start	End
174	189	499	636	769	806	1005	1045
174	190	499	637	769	809	1005	1046
174	193	499	638	769	810	1005	1047
174	195	499	641	769	811	1005	1049
174	197	499	642	769	819	1005	1050
174	198	499	643	769	823	1005	1051
174	199	499	646	769	825	1005	1053
174	200	499	647	769	826	1005	1055
174	201	499	648	769	827	1005	1056
174	203	499	649	769	831	1005	1057
174	205	499	650	769	832	1005	1058
174	206	499	651	769	833	1005	1059
174	207	499	652	769	835	1005	1061
174	209	499	654	769	836	1005	1062
174	210	499	658	769	837	1005	1064
174	211	499	659	769	839	1005	1065
174	212	499	660	769	841	1005	1066
174	213	499	661	769	843	1005	1067
174	214	499	664	769	845	1005	1068
174	215	499	665	769	848	1005	1069
174	216	499	666	769	850	1005	1070
174	218	499	667	769	851	1005	1071

FIG. 23

174	219	499	669	769	853	1005	1072
174	220	499	670	769	855	1005	1073
174	222	499	671	769	856	1005	1074
174	223	499	672	769	858	1005	1075
174	224	499	673	769	859	1005	1076
174	226	499	674	769	860	1005	1077
174	227	499	675	769	862	1005	1078
174	228	499	677	769	863	1005	1079
174	229	499	679	769	865	1005	1080
174	230	499	680	769	866	1005	1081
174	231	499	683	769	868	1005	1082
174	232	499	684	769	870	1005	1083
174	233	499	686	769	871	1005	1084
174	234	499	687	769	872	1005	1085
174	235	499	692	769	876	1005	1086
174	236	499	700	769	878	1005	1089
174	237	499	701	769	879	1005	1093
174	238	499	702	769	880	1005	1097
174	239	499	703	769	881	1005	1099
174	240	499	704	769	882	1005	1100
174	241	499	705	769	883	1005	1104
174	242	499	708	769	884	1005	1105
174	243	499	709	769	885	1005	1106
174	244	499	710	769	888	1006	1032
174	245	499	712	769	889	1006	1039

FIG. 23

174	246	499	714	769	890	1006	1041
174	247	499	715	769	891	1006	1045
174	248	499	717	769	892	1006	1046
174	249	499	720	769	893	1006	1049
174	250	499	722	769	894	1006	1051
174	251	499	725	769	895	1006	1056
174	252	499	726	769	897	1006	1059
174	253	500	533	769	898	1006	1062
174	254	500	534	769	899	1006	1064
174	255	500	542	769	900	1006	1065
174	257	500	545	769	901	1006	1067
174	258	500	558	769	902	1006	1068
174	259	500	566	769	903	1006	1069
174	260	500	571	769	904	1006	1071
174	261	500	572	769	905	1006	1072
174	263	500	573	769	906	1006	1073
174	264	500	590	769	907	1006	1074
174	265	500	598	769	908	1006	1075
174	266	500	604	769	910	1006	1076
174	267	500	606	769	911	1006	1077
174	268	500	609	769	912	1006	1078
174	271	500	620	769	913	1006	1079
174	273	500	625	769	915	1006	1080
174	274	500	626	769	916	1006	1081
174	275	500	631	769	917	1006	1083

FIG. 23

174	276	500	633	769	918	1006	1084
174	277	500	638	769	919	1006	1085
174	278	500	641	769	920	1006	1086
174	279	500	642	769	921	1006	1094
174	280	500	643	769	922	1006	1108
174	281	500	644	769	923	1007	1026
174	283	500	645	769	924	1007	1029
174	284	500	646	769	925	1007	1038
174	285	500	647	769	927	1007	1039
174	287	500	648	769	928	1007	1043
174	288	500	649	769	929	1007	1044
174	289	500	650	769	931	1007	1045
174	290	500	651	769	932	1007	1046
174	291	500	652	769	933	1007	1051
174	292	500	653	769	935	1007	1056
174	294	500	654	769	937	1007	1058
174	295	500	655	769	941	1007	1059
174	296	500	656	769	942	1007	1061
174	297	500	658	769	948	1007	1062
174	300	500	660	769	949	1007	1064
174	301	500	661	769	950	1007	1065
174	303	500	662	769	951	1007	1066
174	304	500	664	769	956	1007	1067
174	305	500	665	769	958	1007	1068
174	306	500	667	769	959	1007	1069

FIG. 23

174	307	500	668	769	960	1007	1070
174	308	500	671	769	961	1007	1071
174	311	500	672	769	964	1007	1072
174	312	500	673	769	966	1007	1074
174	313	500	674	769	969	1007	1075
174	314	500	675	769	971	1007	1076
174	315	500	677	769	973	1007	1077
174	316	500	678	769	980	1007	1079
174	317	500	679	770	773	1007	1080
174	318	500	680	770	779	1007	1081
174	319	500	681	770	785	1007	1083
174	320	500	682	770	797	1007	1084
174	321	500	683	770	799	1007	1086
174	324	500	684	770	803	1007	1095
175	181	500	685	770	810	1007	1097
175	182	500	686	770	813	1007	1099
175	183	500	687	770	819	1007	1102
175	185	500	688	770	822	1007	1105
175	187	500	689	770	823	1007	1108
175	191	500	692	770	826	1007	1111
175	193	500	697	770	830	1008	1029
175	195	500	699	770	831	1008	1032
175	198	500	701	770	832	1008	1037
175	199	500	702	770	834	1008	1039
175	203	500	703	770	835	1008	1040

FIG. 23

175	204	500	704	770	837	1008	1041
175	208	500	705	770	839	1008	1043
175	210	500	706	770	840	1008	1044
175	211	500	708	770	841	1008	1045
175	214	500	710	770	843	1008	1046
175	215	500	711	770	848	1008	1047
175	216	500	712	770	849	1008	1048
175	217	500	713	770	851	1008	1049
175	218	500	714	770	853	1008	1051
175	220	500	715	770	854	1008	1056
175	222	500	717	770	855	1008	1058
175	223	500	718	770	856	1008	1061
175	224	500	720	770	857	1008	1062
175	226	500	721	770	858	1008	1064
175	227	500	722	770	859	1008	1065
175	228	500	723	770	860	1008	1066
175	229	500	724	770	862	1008	1067
175	231	500	725	770	867	1008	1068
175	232	500	727	770	869	1008	1069
175	233	500	728	770	870	1008	1070
175	234	500	729	770	871	1008	1071
175	235	501	537	770	879	1008	1072
175	236	501	543	770	880	1008	1073
175	237	501	544	770	881	1008	1074
175	239	501	545	770	882	1008	1075

FIG. 23

175	240	501	546	770	883	1008	1076
175	241	501	562	770	884	1008	1077
175	242	501	572	770	885	1008	1078
175	243	501	573	770	889	1008	1079
175	244	501	583	770	890	1008	1080
175	245	501	585	770	891	1008	1081
175	246	501	586	770	892	1008	1082
175	247	501	589	770	893	1008	1083
175	248	501	592	770	894	1008	1084
175	249	501	604	770	895	1008	1085
175	250	501	609	770	897	1008	1089
175	251	501	611	770	898	1008	1090
175	253	501	613	770	899	1008	1092
175	254	501	620	770	900	1008	1093
175	255	501	621	770	901	1008	1096
175	257	501	626	770	902	1008	1097
175	262	501	628	770	903	1008	1103
175	263	501	631	770	904	1008	1104
175	264	501	635	770	905	1009	1028
175	265	501	636	770	906	1009	1029
175	266	501	637	770	907	1009	1030
175	267	501	638	770	908	1009	1031
175	268	501	640	770	909	1009	1032
175	271	501	641	770	910	1009	1033
175	272	501	642	770	911	1009	1037

FIG. 23

175	273	501	643	770	912	1009	1039
175	274	501	644	770	913	1009	1040
175	275	501	645	770	915	1009	1042
175	276	501	646	770	916	1009	1043
175	278	501	647	770	917	1009	1044
175	280	501	648	770	918	1009	1045
175	281	501	649	770	919	1009	1046
175	283	501	650	770	920	1009	1047
175	284	501	651	770	921	1009	1049
175	285	501	652	770	922	1009	1051
175	287	501	653	770	923	1009	1053
175	288	501	654	770	924	1009	1055
175	289	501	656	770	925	1009	1056
175	290	501	657	770	926	1009	1057
175	291	501	658	770	927	1009	1058
175	292	501	659	770	928	1009	1059
175	293	501	660	770	929	1009	1061
175	294	501	661	770	931	1009	1062
175	295	501	662	770	932	1009	1064
175	296	501	663	770	933	1009	1065
175	297	501	664	770	934	1009	1066
175	299	501	665	770	939	1009	1067
175	300	501	666	770	940	1009	1068
175	301	501	667	770	941	1009	1069
175	303	501	668	770	947	1009	1070

FIG. 23

175	305	501	669	770	948	1009	1071
175	306	501	670	770	950	1009	1072
175	307	501	671	770	951	1009	1073
175	308	501	672	770	953	1009	1074
175	309	501	673	770	955	1009	1075
175	310	501	674	770	956	1009	1076
175	311	501	675	770	957	1009	1077
175	312	501	677	770	958	1009	1078
175	313	501	678	770	959	1009	1079
175	315	501	679	770	960	1009	1080
175	316	501	680	770	961	1009	1081
175	317	501	681	770	962	1009	1082
175	318	501	682	770	964	1009	1083
175	319	501	683	770	965	1009	1084
175	320	501	684	770	973	1009	1085
175	321	501	685	770	980	1009	1086
175	323	501	686	771	776	1009	1087
175	324	501	687	771	785	1009	1088
176	185	501	688	771	792	1009	1093
176	195	501	689	771	797	1009	1094
176	205	501	690	771	806	1009	1096
176	212	501	692	771	811	1009	1097
176	214	501	695	771	821	1009	1104
176	215	501	696	771	824	1009	1106
176	216	501	697	771	826	1009	1107

FIG. 23

176	217	501	698	771	830	1009	1110
176	220	501	699	771	831	1009	1111
176	221	501	700	771	833	1009	1112
176	223	501	701	771	844	1010	1029
176	227	501	702	771	848	1010	1030
176	228	501	703	771	851	1010	1032
176	229	501	704	771	853	1010	1033
176	231	501	705	771	854	1010	1035
176	233	501	706	771	855	1010	1036
176	234	501	708	771	856	1010	1037
176	235	501	709	771	857	1010	1038
176	237	501	710	771	867	1010	1039
176	238	501	711	771	868	1010	1040
176	241	501	712	771	880	1010	1041
176	242	501	713	771	881	1010	1042
176	243	501	714	771	882	1010	1043
176	244	501	715	771	890	1010	1044
176	245	501	716	771	892	1010	1045
176	250	501	717	771	898	1010	1046
176	253	501	719	771	900	1010	1047
176	255	501	720	771	901	1010	1048
176	256	501	721	771	904	1010	1049
176	260	501	722	771	906	1010	1050
176	261	501	723	771	908	1010	1051
176	264	501	724	771	909	1010	1053

FIG. 23

176	265	501	725	771	910	1010	1055
176	271	501	726	771	911	1010	1056
176	272	501	727	771	912	1010	1057
176	274	501	728	771	913	1010	1058
176	275	501	729	771	916	1010	1059
176	276	501	730	771	917	1010	1060
176	278	501	737	771	919	1010	1061
176	279	502	533	771	920	1010	1062
176	284	502	543	771	921	1010	1063
176	287	502	544	771	923	1010	1064
176	289	502	545	771	924	1010	1065
176	291	502	548	771	925	1010	1066
176	294	502	568	771	933	1010	1067
176	295	502	576	771	937	1010	1068
176	296	502	587	771	938	1010	1069
176	297	502	589	771	949	1010	1070
176	299	502	598	771	951	1010	1071
176	302	502	600	771	952	1010	1072
176	306	502	611	771	956	1010	1073
176	312	502	621	771	959	1010	1074
176	313	502	622	771	968	1010	1075
176	314	502	624	771	973	1010	1076
176	315	502	626	771	974	1010	1077
176	316	502	630	772	782	1010	1078
176	317	502	631	772	789	1010	1079

FIG. 23

176	318	502	632	772	803	1010	1080
176	319	502	637	772	804	1010	1081
176	321	502	641	772	808	1010	1082
177	181	502	642	772	809	1010	1083
177	210	502	643	772	810	1010	1084
177	211	502	644	772	826	1010	1085
177	214	502	645	772	832	1010	1086
177	216	502	646	772	841	1010	1087
177	217	502	647	772	852	1010	1088
177	222	502	648	772	856	1010	1091
177	223	502	649	772	857	1010	1094
177	227	502	650	772	858	1010	1095
177	229	502	652	772	859	1010	1107
177	233	502	653	772	860	1011	1058
177	236	502	654	772	869	1011	1062
177	242	502	655	772	871	1011	1064
177	243	502	656	772	879	1011	1066
177	244	502	658	772	880	1011	1067
177	245	502	659	772	881	1011	1068
177	247	502	660	772	882	1011	1075
177	250	502	661	772	883	1011	1080
177	253	502	662	772	890	1011	1081
177	255	502	664	772	893	1011	1083
177	260	502	665	772	895	1011	1084
177	265	502	667	772	898	1011	1089

FIG. 23

177	272	502	668	772	899	1012	1032
177	276	502	670	772	901	1012	1033
177	278	502	671	772	902	1012	1036
177	284	502	672	772	904	1012	1037
177	287	502	673	772	906	1012	1038
177	290	502	674	772	907	1012	1039
177	294	502	675	772	908	1012	1042
177	295	502	677	772	910	1012	1044
177	297	502	679	772	913	1012	1045
177	300	502	680	772	914	1012	1046
177	301	502	681	772	916	1012	1047
177	306	502	682	772	917	1012	1048
177	309	502	683	772	918	1012	1049
177	312	502	684	772	919	1012	1051
177	316	502	685	772	920	1012	1053
177	318	502	686	772	921	1012	1055
177	319	502	687	772	923	1012	1056
177	321	502	688	772	924	1012	1057
178	182	502	690	772	925	1012	1058
178	185	502	692	772	928	1012	1059
178	189	502	693	772	929	1012	1061
178	203	502	695	772	932	1012	1062
178	205	502	697	772	933	1012	1063
178	211	502	698	772	937	1012	1064
178	213	502	699	772	950	1012	1065

FIG. 23

178	216	502	701	772	955	1012	1066
178	220	502	702	772	957	1012	1067
178	224	502	703	772	958	1012	1068
178	226	502	706	772	959	1012	1069
178	227	502	708	772	961	1012	1070
178	228	502	709	772	968	1012	1071
178	232	502	710	772	971	1012	1072
178	235	502	712	772	973	1012	1073
178	242	502	713	772	980	1012	1074
178	243	502	714	772	981	1012	1075
178	244	502	715	773	835	1012	1076
178	245	502	717	773	882	1012	1077
178	246	502	719	773	883	1012	1078
178	250	502	720	773	897	1012	1079
178	255	502	721	773	901	1012	1080
178	260	502	722	773	905	1012	1081
178	261	502	723	773	915	1012	1082
178	271	502	724	773	918	1012	1083
178	272	502	725	773	919	1012	1084
178	273	502	726	773	924	1012	1085
178	275	502	728	773	925	1012	1086
178	276	502	729	773	971	1012	1087
178	277	502	730	774	785	1012	1088
178	284	502	732	774	797	1012	1094
178	285	503	534	774	803	1012	1096

FIG. 23

178	287	503	536	774	810	1012	1103
178	290	503	537	774	817	1012	1105
178	293	503	540	774	821	1012	1108
178	295	503	550	774	826	1012	1110
178	297	503	553	774	830	1013	1039
178	300	503	558	774	842	1013	1044
178	301	503	561	774	843	1013	1046
178	305	503	568	774	848	1013	1047
178	306	503	573	774	850	1013	1053
178	308	503	574	774	851	1013	1058
178	312	503	589	774	854	1013	1062
178	313	503	590	774	857	1013	1065
178	314	503	592	774	859	1013	1066
178	318	503	594	774	860	1013	1067
178	319	503	595	774	868	1013	1068
178	321	503	596	774	871	1013	1069
179	185	503	598	774	880	1013	1071
179	188	503	603	774	881	1013	1072
179	189	503	604	774	885	1013	1073
179	190	503	607	774	891	1013	1074
179	193	503	615	774	892	1013	1075
179	195	503	617	774	897	1013	1081
179	196	503	620	774	898	1013	1083
179	197	503	623	774	901	1013	1084
179	200	503	626	774	904	1013	1085

FIG. 23

179	201	503	628	774	906	1013	1088
179	206	503	630	774	911	1013	1108
179	210	503	631	774	912	1013	1111
179	211	503	632	774	916	1014	1058
179	212	503	635	774	917	1014	1064
179	213	503	636	774	919	1014	1073
179	214	503	638	774	920	1014	1074
179	217	503	639	774	921	1014	1075
179	218	503	640	774	923	1014	1083
179	219	503	641	774	924	1014	1085
179	220	503	642	774	925	1014	1089
179	222	503	643	774	928	1014	1103
179	224	503	644	774	933	1015	1037
179	226	503	645	774	937	1015	1038
179	227	503	646	774	941	1015	1039
179	228	503	647	774	948	1015	1040
179	229	503	648	774	951	1015	1041
179	230	503	649	774	955	1015	1047
179	232	503	650	774	956	1015	1049
179	233	503	651	774	958	1015	1053
179	234	503	652	774	959	1015	1055
179	235	503	653	774	968	1015	1056
179	236	503	654	774	970	1015	1057
179	237	503	655	774	971	1015	1058
179	239	503	656	775	782	1015	1059

FIG. 23

179	240	503	657	775	786	1015	1061
179	242	503	658	775	792	1015	1062
179	243	503	659	775	824	1015	1064
179	244	503	660	775	826	1015	1065
179	245	503	661	775	829	1015	1066
179	246	503	662	775	854	1015	1067
179	247	503	663	775	856	1015	1068
179	249	503	664	775	880	1015	1069
179	250	503	665	775	892	1015	1070
179	251	503	666	775	897	1015	1071
179	254	503	667	775	898	1015	1072
179	255	503	668	775	900	1015	1074
179	259	503	669	775	901	1015	1075
179	261	503	670	775	906	1015	1076
179	264	503	671	775	910	1015	1077
179	265	503	672	775	916	1015	1078
179	266	503	673	775	919	1015	1079
179	269	503	674	775	920	1015	1080
179	271	503	675	775	922	1015	1081
179	272	503	677	775	924	1015	1082
179	274	503	678	775	925	1015	1083
179	275	503	679	775	933	1015	1084
179	276	503	680	775	949	1015	1085
179	277	503	681	775	951	1015	1086
179	278	503	682	775	961	1015	1090

FIG. 23

179	280	503	683	775	968	1015	1096
179	281	503	684	775	973	1015	1099
179	283	503	685	775	980	1015	1103
179	284	503	686	776	790	1015	1106
179	285	503	687	776	826	1015	1111
179	286	503	688	776	827	1015	1112
179	287	503	689	776	834	1016	1039
179	289	503	691	776	851	1016	1055
179	291	503	692	776	855	1016	1056
179	292	503	693	776	856	1016	1058
179	294	503	695	776	858	1016	1059
179	295	503	696	776	860	1016	1062
179	296	503	697	776	863	1016	1068
179	297	503	698	776	871	1016	1069
179	299	503	699	776	880	1016	1071
179	301	503	700	776	881	1016	1073
179	303	503	701	776	884	1016	1074
179	305	503	702	776	886	1016	1076
179	306	503	703	776	889	1016	1077
179	307	503	704	776	890	1016	1080
179	308	503	705	776	892	1016	1081
179	309	503	706	776	898	1016	1104
179	311	503	707	776	899	1017	1038
179	312	503	708	776	900	1017	1039
179	313	503	709	776	901	1017	1041

FIG. 23

179	314	503	710	776	902	1017	1042
179	315	503	711	776	904	1017	1043
179	316	503	713	776	905	1017	1045
179	317	503	714	776	906	1017	1047
179	318	503	715	776	908	1017	1048
179	319	503	716	776	911	1017	1056
179	321	503	717	776	912	1017	1057
180	184	503	718	776	913	1017	1059
180	189	503	719	776	915	1017	1062
180	193	503	720	776	916	1017	1064
180	194	503	721	776	917	1017	1065
180	195	503	722	776	920	1017	1066
180	196	503	723	776	921	1017	1067
180	197	503	724	776	922	1017	1068
180	198	503	725	776	924	1017	1070
180	207	503	726	776	925	1017	1071
180	208	503	728	776	926	1017	1072
180	211	503	729	776	929	1017	1073
180	212	503	730	776	932	1017	1074
180	214	503	731	776	941	1017	1075
180	216	503	734	776	942	1017	1076
180	217	504	535	776	951	1017	1077
180	220	504	538	776	958	1017	1078
180	222	504	544	776	959	1017	1079
180	223	504	545	776	961	1017	1080

FIG. 23

180	224	504	568	776	966	1017	1081
180	225	504	572	776	971	1017	1082
180	226	504	573	776	980	1017	1083
180	227	504	606	777	785	1017	1084
180	228	504	609	777	795	1017	1086
180	229	504	617	777	796	1017	1087
180	231	504	626	777	806	1017	1088
180	233	504	640	777	813	1017	1095
180	234	504	641	777	816	1017	1108
180	235	504	642	777	819	1017	1110
180	236	504	643	777	822	1017	1112
180	237	504	644	777	827	1018	1039
180	238	504	645	777	831	1018	1051
180	239	504	646	777	832	1018	1058
180	242	504	647	777	835	1018	1065
180	243	504	648	777	836	1018	1067
180	244	504	649	777	839	1018	1069
180	245	504	650	777	842	1018	1075
180	246	504	651	777	843	1018	1077
180	247	504	652	777	844	1018	1079
180	248	504	653	777	845	1018	1080
180	249	504	654	777	846	1018	1081
180	250	504	655	777	848	1018	1085
180	251	504	658	777	851	1018	1087
180	252	504	660	777	853	1018	1106

FIG. 23

180	253	504	661	777	854	1019	1038
180	255	504	662	777	855	1019	1039
180	257	504	663	777	857	1019	1051
180	260	504	664	777	858	1019	1058
180	263	504	665	777	860	1019	1059
180	264	504	666	777	863	1019	1063
180	265	504	667	777	866	1019	1066
180	271	504	668	777	868	1019	1067
180	272	504	669	777	869	1019	1068
180	273	504	671	777	871	1019	1069
180	274	504	672	777	873	1019	1071
180	275	504	673	777	877	1019	1072
180	276	504	674	777	878	1019	1073
180	277	504	675	777	879	1019	1074
180	279	504	676	777	881	1019	1075
180	283	504	677	777	882	1019	1076
180	284	504	679	777	884	1019	1077
180	285	504	680	777	885	1019	1080
180	286	504	683	777	886	1019	1081
180	287	504	684	777	890	1019	1084
180	289	504	685	777	891	1019	1085
180	290	504	686	777	892	1019	1087
180	291	504	687	777	893	1019	1088
180	292	504	688	777	894	1019	1089
180	294	504	697	777	898	1019	1104

FIG. 23

180	295	504	698	777	899	1019	1105
180	296	504	699	777	901	1020	1039
180	297	504	700	777	902	1020	1043
180	300	504	702	777	903	1020	1044
180	302	504	703	777	904	1020	1046
180	304	504	704	777	905	1020	1047
180	305	504	705	777	906	1020	1051
180	306	504	706	777	908	1020	1053
180	307	504	708	777	910	1020	1058
180	308	504	709	777	912	1020	1064
180	311	504	710	777	915	1020	1065
180	312	504	712	777	916	1020	1066
180	315	504	713	777	917	1020	1067
180	316	504	715	777	918	1020	1069
180	317	504	716	777	919	1020	1070
180	318	504	717	777	920	1020	1071
180	319	504	718	777	921	1020	1072
180	320	504	720	777	922	1020	1074
180	321	504	721	777	923	1020	1075
181	185	504	722	777	924	1020	1077
181	186	504	723	777	925	1020	1078
181	211	504	724	777	926	1020	1080
181	216	504	725	777	927	1020	1081
181	220	504	727	777	928	1020	1083
181	228	504	728	777	929	1020	1084

FIG. 23

181	229	504	729	777	931	1020	1089
181	232	504	730	777	932	1020	1101
181	233	504	731	777	934	1020	1108
181	242	504	732	777	938	1020	1112
181	243	504	734	777	940	1021	1066
181	244	505	537	777	942	1021	1067
181	245	505	544	777	947	1021	1068
181	246	505	551	777	948	1021	1070
181	249	505	562	777	950	1021	1072
181	250	505	613	777	951	1021	1074
181	253	505	621	777	953	1021	1075
181	255	505	630	777	954	1021	1080
181	257	505	631	777	955	1021	1087
181	261	505	641	777	956	1022	1042
181	271	505	643	777	957	1022	1051
181	272	505	646	777	958	1022	1053
181	285	505	647	777	959	1022	1055
181	286	505	648	777	960	1022	1056
181	287	505	652	777	961	1022	1060
181	288	505	654	777	962	1022	1064
181	291	505	656	777	963	1022	1065
181	294	505	657	777	964	1022	1066
181	295	505	658	777	969	1022	1067
181	296	505	659	777	970	1022	1068
181	297	505	660	777	971	1022	1069

FIG. 23

181	301	505	661	777	973	1022	1071
181	302	505	663	777	977	1022	1072
181	306	505	664	777	979	1022	1074
181	311	505	665	778	810	1022	1075
181	312	505	667	778	823	1022	1076
181	316	505	670	778	824	1022	1078
181	318	505	671	778	826	1022	1083
181	319	505	672	778	835	1022	1084
181	320	505	673	778	843	1022	1085
181	321	505	675	778	851	1022	1086
182	191	505	677	778	856	1022	1087
182	200	505	679	778	857	1022	1096
182	211	505	680	778	869	1022	1103
182	216	505	682	778	882	1022	1104
182	228	505	683	778	885	1023	1044
182	230	505	684	778	890	1023	1061
182	231	505	686	778	892	1023	1067
182	242	505	687	778	895	1023	1068
182	243	505	688	778	898	1023	1072
182	244	505	689	778	900	1023	1083
182	250	505	692	778	901	1023	1105
182	255	505	699	778	904	1024	1044
182	272	505	700	778	906	1024	1045
182	273	505	701	778	909	1024	1046
182	274	505	702	778	912	1024	1047

FIG. 23

182	275	505	703	778	915	1024	1048
182	276	505	704	778	916	1024	1049
182	278	505	708	778	917	1024	1053
182	279	505	709	778	918	1024	1056
182	284	505	710	778	920	1024	1058
182	287	505	711	778	921	1024	1059
182	295	505	713	778	922	1024	1061
182	297	505	714	778	923	1024	1062
182	300	505	715	778	924	1024	1063
182	301	505	718	778	925	1024	1065
182	305	505	719	778	932	1024	1066
182	312	505	720	778	948	1024	1067
182	314	505	722	778	950	1024	1068
182	318	505	725	778	951	1024	1069
182	319	505	726	778	955	1024	1070
182	321	505	727	778	959	1024	1071
182	324	505	728	778	961	1024	1072
183	200	505	729	778	964	1024	1073
183	213	505	730	778	968	1024	1074
183	215	505	731	778	980	1024	1075
183	216	505	732	779	853	1024	1076
183	234	506	545	779	885	1024	1077
183	238	506	547	779	892	1024	1078
183	242	506	548	779	894	1024	1080
183	243	506	572	779	899	1024	1081

FIG. 23

183	250	506	623	779	901	1024	1085
183	271	506	625	779	907	1024	1086
183	274	506	626	779	916	1024	1087
183	284	506	643	779	924	1024	1090
183	287	506	644	779	925	1024	1091
183	295	506	645	779	928	1024	1104
183	302	506	647	779	934	1024	1110
183	305	506	650	779	964	1025	1044
183	306	506	651	779	973	1025	1045
183	314	506	654	779	977	1025	1046
183	321	506	655	780	785	1025	1047
184	200	506	658	780	791	1025	1058
184	205	506	660	780	810	1025	1059
184	211	506	664	780	814	1025	1062
184	213	506	665	780	819	1025	1067
184	216	506	667	780	823	1025	1068
184	217	506	671	780	826	1025	1069
184	222	506	672	780	831	1025	1070
184	224	506	673	780	840	1025	1071
184	228	506	675	780	843	1025	1072
184	229	506	680	780	848	1025	1073
184	230	506	684	780	849	1025	1074
184	231	506	685	780	851	1025	1075
184	234	506	686	780	857	1025	1077
184	235	506	687	780	859	1025	1081

FIG. 23

184	242	506	688	780	860	1025	1083
184	245	506	699	780	868	1025	1084
184	246	506	702	780	881	1025	1087
184	250	506	704	780	885	1025	1089
184	253	506	705	780	892	1025	1090
184	260	506	708	780	893	1025	1091
184	261	506	715	780	895	1025	1094
184	271	506	716	780	897	1025	1096
184	272	506	717	780	898	1025	1105
184	273	506	721	780	900	1025	1111
184	274	506	723	780	901	1026	1072
184	276	506	724	780	902	1026	1075
184	283	506	725	780	904	1026	1080
184	287	506	728	780	909	1026	1081
184	290	506	729	780	910	1026	1088
184	295	506	730	780	916	1026	1090
184	297	506	732	780	917	1026	1095
184	300	506	734	780	918	1027	1046
184	305	507	545	780	919	1027	1055
184	306	507	548	780	920	1027	1057
184	312	507	572	780	921	1027	1058
184	314	507	589	780	922	1027	1062
184	318	507	606	780	923	1027	1064
184	321	507	609	780	924	1027	1065
184	323	507	626	780	925	1027	1066

FIG. 23

185	190	507	635	780	928	1027	1067
185	196	507	638	780	933	1027	1068
185	197	507	641	780	934	1027	1069
185	198	507	643	780	956	1027	1071
185	200	507	644	780	968	1027	1072
185	204	507	645	780	970	1027	1073
185	205	507	646	780	971	1027	1074
185	211	507	647	781	797	1027	1075
185	216	507	648	781	827	1027	1076
185	217	507	650	781	833	1027	1077
185	223	507	651	781	842	1027	1080
185	226	507	653	781	846	1027	1081
185	227	507	654	781	852	1027	1083
185	228	507	659	781	854	1027	1086
185	229	507	660	781	855	1027	1087
185	230	507	661	781	860	1027	1088
185	231	507	664	781	867	1027	1090
185	233	507	665	781	870	1027	1095
185	235	507	670	781	871	1027	1105
185	240	507	671	781	875	1027	1112
185	242	507	672	781	879	1028	1053
185	243	507	673	781	880	1028	1058
185	244	507	674	781	882	1028	1062
185	245	507	675	781	884	1028	1065
185	246	507	676	781	885	1028	1066

FIG. 23

185	247	507	678	781	886	1028	1067
185	248	507	683	781	888	1028	1068
185	249	507	684	781	892	1028	1069
185	250	507	685	781	897	1028	1074
185	253	507	686	781	898	1028	1075
185	255	507	687	781	900	1028	1079
185	263	507	688	781	903	1028	1081
185	264	507	689	781	906	1028	1085
185	265	507	697	781	907	1028	1087
185	272	507	699	781	910	1028	1089
185	273	507	700	781	915	1028	1090
185	274	507	701	781	918	1029	1048
185	275	507	703	781	919	1029	1053
185	278	507	704	781	920	1029	1057
185	284	507	705	781	921	1029	1058
185	287	507	706	781	922	1029	1065
185	289	507	709	781	923	1029	1066
185	290	507	713	781	924	1029	1067
185	294	507	716	781	925	1029	1068
185	295	507	717	781	928	1029	1069
185	296	507	720	781	929	1029	1072
185	297	507	721	781	932	1029	1073
185	305	507	722	781	950	1029	1074
185	306	507	724	781	953	1029	1075
185	309	507	725	781	958	1029	1076

FIG. 23

185	312	507	728	781	961	1029	1077
185	315	507	729	781	963	1029	1078
185	316	507	730	781	964	1029	1081
185	321	507	731	781	969	1029	1083
185	323	507	732	781	971	1029	1087
185	324	508	544	781	973	1029	1090
186	190	508	545	781	977	1029	1101
186	194	508	546	781	980	1029	1106
186	198	508	547	782	796	1029	1108
186	199	508	548	782	797	1029	1111
186	207	508	561	782	814	1030	1058
186	208	508	563	782	822	1030	1059
186	214	508	572	782	835	1030	1064
186	217	508	598	782	845	1030	1067
186	220	508	603	782	851	1030	1068
186	221	508	606	782	855	1030	1069
186	222	508	609	782	857	1030	1070
186	223	508	613	782	883	1030	1071
186	224	508	621	782	884	1030	1072
186	227	508	625	782	887	1030	1074
186	228	508	626	782	891	1030	1075
186	229	508	629	782	892	1030	1078
186	230	508	630	782	903	1030	1080
186	231	508	632	782	904	1030	1081
186	232	508	641	782	909	1030	1082

FIG. 23

186	233	508	643	782	916	1030	1083
186	234	508	644	782	919	1030	1084
186	238	508	645	782	920	1030	1085
186	239	508	646	782	923	1030	1086
186	240	508	647	782	924	1030	1087
186	241	508	648	782	925	1030	1089
186	242	508	649	782	929	1030	1090
186	243	508	650	782	932	1030	1094
186	244	508	651	782	933	1030	1106
186	245	508	652	782	964	1030	1112
186	246	508	653	782	968	1031	1051
186	247	508	654	782	974	1031	1058
186	248	508	655	783	819	1031	1062
186	250	508	656	783	821	1031	1064
186	251	508	657	783	822	1031	1065
186	252	508	658	783	826	1031	1067
186	253	508	660	783	832	1031	1072
186	254	508	661	783	833	1031	1074
186	255	508	662	783	835	1031	1075
186	256	508	665	783	838	1031	1078
186	258	508	667	783	839	1031	1080
186	263	508	668	783	846	1031	1081
186	264	508	671	783	850	1031	1083
186	265	508	672	783	852	1031	1084
186	266	508	673	783	854	1031	1089

FIG. 23

186	267	508	674	783	855	1031	1090
186	270	508	675	783	858	1031	1091
186	272	508	676	783	860	1031	1097
186	274	508	677	783	861	1031	1106
186	275	508	678	783	870	1032	1053
186	277	508	679	783	871	1032	1061
186	278	508	683	783	876	1032	1062
186	279	508	684	783	882	1032	1067
186	281	508	685	783	883	1032	1071
186	283	508	686	783	886	1032	1072
186	284	508	687	783	891	1032	1073
186	285	508	688	783	892	1032	1074
186	287	508	690	783	893	1032	1075
186	288	508	693	783	894	1032	1079
186	289	508	697	783	899	1032	1087
186	291	508	698	783	901	1032	1089
186	292	508	699	783	904	1032	1091
186	294	508	700	783	906	1032	1103
186	295	508	701	783	908	1033	1053
186	296	508	702	783	910	1033	1058
186	297	508	703	783	915	1033	1061
186	300	508	704	783	918	1033	1062
186	301	508	705	783	920	1033	1064
186	305	508	706	783	921	1033	1065
186	306	508	708	783	922	1033	1066

FIG. 23

186	307	508	709	783	923	1033	1067
186	309	508	710	783	924	1033	1068
186	311	508	711	783	925	1033	1070
186	312	508	712	783	926	1033	1072
186	313	508	713	783	928	1033	1073
186	315	508	714	783	929	1033	1074
186	316	508	715	783	933	1033	1075
186	317	508	716	783	950	1033	1076
186	318	508	717	783	953	1033	1080
186	319	508	718	783	957	1033	1081
186	320	508	719	783	958	1033	1082
186	321	508	720	783	959	1033	1083
186	322	508	722	783	961	1033	1084
186	323	508	723	783	964	1033	1085
186	324	508	724	783	971	1033	1086
187	198	508	725	783	973	1033	1087
187	205	508	726	783	977	1033	1088
187	211	508	727	784	851	1033	1089
187	212	508	728	784	854	1033	1090
187	214	508	729	784	856	1033	1091
187	216	508	730	784	860	1033	1105
187	217	508	731	784	867	1033	1106
187	218	508	732	784	901	1034	1058
187	226	508	738	784	904	1034	1059
187	227	509	538	784	910	1034	1062

FIG. 23

187	228	509	542	784	920	1034	1067
187	229	509	543	784	924	1034	1068
187	232	509	544	784	925	1034	1069
187	233	509	545	785	790	1034	1071
187	239	509	546	785	814	1034	1072
187	243	509	547	785	826	1034	1075
187	244	509	548	785	829	1034	1077
187	245	509	562	785	835	1034	1079
187	249	509	563	785	855	1034	1080
187	260	509	564	785	858	1034	1081
187	262	509	568	785	859	1034	1085
187	263	509	572	785	868	1034	1086
187	265	509	573	785	898	1034	1087
187	272	509	583	785	900	1034	1089
187	273	509	584	785	901	1034	1090
187	275	509	585	785	904	1034	1094
187	284	509	587	785	906	1034	1101
187	291	509	590	785	908	1034	1105
187	295	509	593	785	909	1034	1106
187	296	509	594	785	918	1035	1058
187	305	509	598	785	919	1035	1060
187	307	509	600	785	920	1035	1065
187	309	509	605	785	921	1035	1066
187	315	509	608	785	923	1035	1068
187	317	509	609	785	924	1035	1070

FIG. 23

187	318	509	610	785	929	1035	1072
187	322	509	613	785	932	1035	1075
187	323	509	614	785	937	1035	1076
188	193	509	617	785	943	1035	1081
188	201	509	618	785	961	1035	1083
188	213	509	620	785	966	1035	1084
188	217	509	621	785	968	1035	1087
188	220	509	623	785	979	1035	1090
188	221	509	624	786	795	1035	1091
188	225	509	625	786	796	1035	1110
188	228	509	626	786	797	1035	1111
188	231	509	627	786	799	1036	1066
188	236	509	628	786	800	1036	1069
188	238	509	630	786	802	1036	1075
188	239	509	631	786	804	1036	1077
188	240	509	632	786	805	1036	1084
188	242	509	634	786	806	1036	1088
188	243	509	635	786	809	1037	1056
188	244	509	636	786	810	1037	1057
188	245	509	637	786	819	1037	1058
188	246	509	638	786	822	1037	1061
188	247	509	639	786	824	1037	1062
188	250	509	640	786	826	1037	1064
188	251	509	641	786	827	1037	1065
188	253	509	642	786	828	1037	1066

FIG. 23

188	255	509	643	786	829	1037	1069
188	260	509	644	786	832	1037	1071
188	261	509	645	786	833	1037	1072
188	262	509	646	786	834	1037	1073
188	264	509	647	786	836	1037	1074
188	271	509	648	786	837	1037	1075
188	272	509	649	786	839	1037	1077
188	274	509	650	786	842	1037	1079
188	275	509	651	786	843	1037	1080
188	276	509	652	786	844	1037	1084
188	278	509	653	786	845	1037	1085
188	279	509	654	786	846	1037	1086
188	281	509	655	786	847	1037	1087
188	284	509	656	786	848	1037	1089
188	285	509	657	786	849	1037	1090
188	287	509	658	786	850	1037	1094
188	290	509	659	786	851	1037	1112
188	291	509	660	786	852	1038	1058
188	295	509	661	786	853	1038	1059
188	296	509	662	786	854	1038	1061
188	297	509	663	786	855	1038	1062
188	299	509	664	786	856	1038	1064
188	300	509	665	786	857	1038	1065
188	301	509	666	786	858	1038	1066
188	302	509	667	786	859	1038	1067

FIG. 23

188	305	509	668	786	860	1038	1068
188	306	509	669	786	862	1038	1069
188	312	509	670	786	865	1038	1071
188	313	509	671	786	866	1038	1072
188	316	509	672	786	867	1038	1073
188	318	509	673	786	868	1038	1074
188	319	509	674	786	869	1038	1075
188	321	509	675	786	870	1038	1076
188	323	509	676	786	871	1038	1078
188	324	509	677	786	872	1038	1079
189	203	509	678	786	874	1038	1080
189	231	509	679	786	875	1038	1081
189	232	509	680	786	877	1038	1082
189	242	509	681	786	878	1038	1084
189	244	509	682	786	879	1038	1086
189	248	509	683	786	881	1038	1087
189	250	509	684	786	882	1038	1088
189	253	509	685	786	883	1038	1089
189	261	509	686	786	884	1038	1090
189	266	509	687	786	885	1038	1091
189	271	509	688	786	886	1038	1101
189	272	509	689	786	887	1038	1103
189	283	509	690	786	888	1038	1104
189	284	509	691	786	889	1038	1106
189	287	509	692	786	890	1038	1110

FIG. 23

189	294	509	693	786	891	1039	1058
189	305	509	695	786	892	1039	1059
189	306	509	696	786	893	1039	1064
189	308	509	697	786	894	1039	1065
189	316	509	698	786	896	1039	1066
189	319	509	699	786	897	1039	1067
190	195	509	700	786	898	1039	1068
190	199	509	701	786	899	1039	1069
190	200	509	702	786	900	1039	1070
190	204	509	703	786	901	1039	1071
190	211	509	704	786	902	1039	1073
190	213	509	705	786	903	1039	1074
190	214	509	706	786	904	1039	1075
190	215	509	707	786	905	1039	1076
190	216	509	708	786	906	1039	1077
190	217	509	709	786	907	1039	1078
190	218	509	710	786	908	1039	1079
190	224	509	711	786	909	1039	1080
190	226	509	712	786	910	1039	1081
190	227	509	713	786	911	1039	1083
190	228	509	714	786	912	1039	1087
190	229	509	715	786	913	1039	1088
190	230	509	716	786	915	1039	1089
190	231	509	717	786	916	1039	1090
190	232	509	718	786	917	1039	1091

FIG. 23

190	233	509	719	786	918	1039	1094
190	235	509	720	786	919	1039	1095
190	239	509	721	786	920	1039	1098
190	242	509	722	786	921	1039	1100
190	243	509	723	786	922	1039	1101
190	244	509	724	786	923	1039	1105
190	245	509	725	786	924	1039	1111
190	246	509	726	786	925	1040	1059
190	247	509	727	786	926	1040	1060
190	250	509	728	786	927	1040	1061
190	251	509	729	786	928	1040	1062
190	253	509	730	786	929	1040	1064
190	254	509	731	786	930	1040	1065
190	255	509	732	786	931	1040	1066
190	257	509	733	786	932	1040	1067
190	271	509	734	786	933	1040	1068
190	272	510	546	786	934	1040	1069
190	275	510	548	786	935	1040	1070
190	276	510	550	786	937	1040	1071
190	279	510	568	786	942	1040	1072
190	287	510	572	786	945	1040	1073
190	292	510	576	786	947	1040	1074
190	295	510	592	786	948	1040	1075
190	296	510	595	786	949	1040	1076
190	297	510	626	786	950	1040	1077

FIG. 23

190	300	510	630	786	951	1040	1078
190	304	510	631	786	953	1040	1079
190	305	510	635	786	954	1040	1080
190	306	510	638	786	955	1040	1081
190	307	510	641	786	956	1040	1082
190	312	510	643	786	957	1040	1083
190	314	510	644	786	958	1040	1084
190	315	510	645	786	959	1040	1085
190	316	510	646	786	960	1040	1086
190	318	510	647	786	961	1040	1087
190	319	510	648	786	963	1040	1088
190	320	510	649	786	964	1040	1089
190	321	510	650	786	965	1040	1090
190	324	510	651	786	967	1040	1091
191	195	510	652	786	968	1040	1102
191	196	510	653	786	969	1040	1104
191	197	510	654	786	970	1040	1105
191	199	510	658	786	971	1040	1110
191	203	510	660	786	973	1040	1111
191	204	510	661	786	975	1040	1112
191	206	510	662	786	977	1041	1067
191	208	510	664	786	980	1041	1068
191	211	510	665	787	792	1041	1069
191	213	510	666	787	808	1041	1070
191	216	510	667	787	810	1041	1072

FIG. 23

191	217	510	668	787	813	1041	1073
191	222	510	669	787	814	1041	1074
191	226	510	670	787	822	1041	1075
191	228	510	671	787	824	1041	1080
191	232	510	672	787	826	1041	1083
191	235	510	673	787	827	1041	1084
191	237	510	674	787	830	1041	1085
191	239	510	675	787	831	1041	1087
191	242	510	678	787	832	1041	1089
191	243	510	679	787	835	1041	1090
191	244	510	680	787	845	1042	1061
191	245	510	682	787	847	1042	1065
191	246	510	683	787	848	1042	1068
191	247	510	684	787	849	1042	1072
191	248	510	686	787	853	1042	1075
191	250	510	687	787	854	1042	1081
191	253	510	688	787	856	1042	1087
191	255	510	689	787	857	1042	1090
191	257	510	690	787	859	1043	1072
191	260	510	692	787	860	1043	1074
191	261	510	696	787	863	1043	1085
191	264	510	697	787	869	1043	1087
191	265	510	701	787	871	1043	1089
191	267	510	702	787	878	1043	1090
191	271	510	703	787	879	1043	1091

FIG. 23

191	272	510	704	787	880	1044	1064
191	273	510	705	787	881	1044	1066
191	274	510	706	787	882	1044	1068
191	275	510	707	787	883	1044	1069
191	277	510	708	787	885	1044	1070
191	283	510	709	787	891	1044	1071
191	284	510	711	787	894	1044	1072
191	285	510	713	787	897	1044	1074
191	287	510	714	787	898	1044	1075
191	291	510	715	787	901	1044	1076
191	293	510	716	787	902	1044	1077
191	294	510	718	787	903	1044	1078
191	295	510	721	787	904	1044	1079
191	296	510	722	787	905	1044	1080
191	297	510	723	787	906	1044	1082
191	301	510	724	787	908	1044	1083
191	305	510	725	787	909	1044	1085
191	306	510	726	787	910	1044	1087
191	308	510	728	787	912	1044	1089
191	311	510	729	787	913	1044	1090
191	312	510	730	787	915	1044	1094
191	313	510	731	787	916	1044	1095
191	315	510	732	787	917	1044	1099
191	316	510	739	787	918	1044	1102
191	317	511	544	787	919	1044	1104

FIG. 23

191	318	511	547	787	920	1044	1112
191	319	511	562	787	921	1045	1065
191	321	511	576	787	922	1045	1066
191	323	511	606	787	923	1045	1067
191	324	511	609	787	924	1045	1068
192	198	511	625	787	925	1045	1069
192	214	511	626	787	926	1045	1070
192	216	511	638	787	929	1045	1071
192	218	511	641	787	932	1045	1072
192	220	511	642	787	933	1045	1073
192	224	511	643	787	950	1045	1074
192	226	511	644	787	951	1045	1075
192	227	511	645	787	955	1045	1076
192	228	511	646	787	960	1045	1077
192	229	511	647	787	961	1045	1078
192	230	511	649	787	964	1045	1079
192	235	511	652	787	965	1045	1080
192	239	511	653	787	968	1045	1081
192	243	511	654	787	971	1045	1083
192	244	511	658	787	979	1045	1084
192	245	511	660	787	981	1045	1085
192	246	511	661	788	817	1045	1087
192	247	511	664	788	824	1045	1088
192	249	511	665	788	833	1045	1089
192	250	511	667	788	856	1045	1090

FIG. 23

192	255	511	668	788	881	1045	1091
192	259	511	671	788	893	1045	1101
192	264	511	672	788	901	1045	1102
192	265	511	673	788	904	1045	1104
192	270	511	675	788	915	1045	1108
192	272	511	676	788	918	1046	1065
192	274	511	680	788	923	1046	1066
192	276	511	681	788	924	1046	1067
192	278	511	684	788	925	1046	1068
192	284	511	685	788	932	1046	1069
192	291	511	686	788	937	1046	1070
192	296	511	687	788	951	1046	1071
192	297	511	688	788	968	1046	1072
192	301	511	689	789	855	1046	1073
192	306	511	697	789	882	1046	1074
192	308	511	698	789	903	1046	1075
192	316	511	699	789	916	1046	1076
192	317	511	700	789	920	1046	1077
192	318	511	701	789	921	1046	1078
192	319	511	702	789	929	1046	1079
192	321	511	703	790	793	1046	1080
192	323	511	704	790	822	1046	1081
192	324	511	705	790	852	1046	1082
193	216	511	706	790	854	1046	1083
193	220	511	707	790	856	1046	1084

FIG. 23

193	222	511	708	790	858	1046	1085
193	228	511	709	790	859	1046	1086
193	229	511	710	790	871	1046	1087
193	232	511	711	790	877	1046	1088
193	234	511	712	790	898	1046	1089
193	235	511	713	790	900	1046	1090
193	237	511	716	790	901	1046	1091
193	250	511	717	790	904	1046	1093
193	253	511	721	790	908	1046	1101
193	276	511	723	790	916	1046	1102
193	284	511	724	790	919	1046	1106
193	289	511	725	790	921	1046	1107
193	295	511	727	790	922	1047	1066
193	297	511	728	790	923	1047	1067
193	308	511	729	790	931	1047	1069
193	311	511	730	790	932	1047	1070
193	312	511	731	790	958	1047	1071
193	318	511	732	790	968	1047	1072
193	319	512	543	790	970	1047	1073
194	198	512	544	790	981	1047	1074
194	200	512	545	791	803	1047	1075
194	201	512	547	791	811	1047	1076
194	204	512	551	791	817	1047	1077
194	205	512	561	791	821	1047	1078
194	210	512	562	791	823	1047	1079

FIG. 23

194	212	512	565	791	827	1047	1080
194	216	512	567	791	831	1047	1081
194	218	512	568	791	834	1047	1083
194	220	512	571	791	849	1047	1084
194	221	512	573	791	851	1047	1085
194	222	512	580	791	852	1047	1086
194	224	512	584	791	855	1047	1087
194	227	512	587	791	856	1047	1088
194	228	512	589	791	861	1047	1089
194	231	512	590	791	864	1047	1090
194	233	512	594	791	877	1047	1091
194	235	512	595	791	881	1047	1101
194	238	512	596	791	882	1047	1111
194	242	512	598	791	883	1048	1067
194	243	512	600	791	885	1048	1068
194	244	512	602	791	888	1048	1071
194	246	512	604	791	892	1048	1072
194	247	512	606	791	895	1048	1073
194	250	512	610	791	898	1048	1076
194	251	512	611	791	899	1048	1077
194	255	512	617	791	900	1048	1078
194	256	512	618	791	901	1048	1080
194	257	512	620	791	904	1048	1081
194	261	512	621	791	908	1048	1083
194	263	512	622	791	909	1048	1084

FIG. 23

194	265	512	624	791	910	1048	1085
194	272	512	625	791	912	1048	1086
194	273	512	626	791	915	1048	1087
194	274	512	627	791	916	1048	1088
194	275	512	628	791	918	1048	1089
194	276	512	630	791	919	1048	1090
194	279	512	631	791	920	1048	1091
194	282	512	632	791	921	1048	1095
194	283	512	634	791	922	1048	1100
194	285	512	635	791	923	1048	1101
194	287	512	636	791	924	1048	1105
194	289	512	637	791	925	1048	1111
194	291	512	638	791	932	1049	1074
194	294	512	639	791	937	1049	1075
194	295	512	640	791	938	1050	1070
194	296	512	641	791	941	1050	1072
194	297	512	642	791	949	1050	1073
194	301	512	643	791	951	1050	1074
194	305	512	644	791	955	1050	1075
194	306	512	645	791	961	1050	1076
194	309	512	646	791	968	1050	1080
194	311	512	647	792	795	1050	1084
194	312	512	648	792	800	1050	1085
194	313	512	649	792	814	1050	1087
194	315	512	650	792	817	1050	1089

FIG. 23

194	316	512	651	792	819	1050	1090
194	317	512	652	792	823	1050	1091
194	318	512	653	792	824	1050	1101
194	319	512	654	792	835	1050	1103
194	320	512	655	792	841	1050	1106
194	321	512	656	792	856	1050	1107
194	322	512	657	792	857	1052	1071
194	323	512	658	792	860	1052	1074
195	201	512	659	792	863	1052	1075
195	203	512	660	792	869	1052	1080
195	211	512	661	792	881	1052	1081
195	216	512	662	792	885	1052	1087
195	217	512	663	792	895	1052	1088
195	223	512	664	792	897	1053	1083
195	224	512	665	792	898	1053	1087
195	227	512	667	792	900	1053	1088
195	228	512	668	792	901	1054	1073
195	229	512	669	792	910	1054	1077
195	231	512	670	792	915	1054	1078
195	232	512	671	792	916	1054	1079
195	233	512	672	792	917	1054	1081
195	240	512	673	792	919	1054	1083
195	242	512	674	792	920	1054	1084
195	243	512	675	792	923	1054	1085
195	245	512	676	792	924	1054	1086

FIG. 23

195	247	512	677	792	925	1054	1087
195	248	512	678	792	932	1054	1088
195	250	512	679	792	933	1054	1089
195	253	512	680	792	937	1054	1090
195	255	512	681	792	938	1054	1094
195	257	512	682	792	941	1054	1098
195	259	512	683	792	947	1054	1099
195	260	512	684	792	949	1054	1104
195	261	512	685	792	961	1054	1106
195	264	512	686	792	968	1054	1110
195	265	512	687	792	971	1055	1089
195	271	512	688	793	797	1056	1077
195	272	512	689	793	810	1056	1078
195	274	512	690	793	814	1056	1087
195	276	512	691	793	819	1056	1088
195	278	512	692	793	858	1056	1089
195	281	512	693	793	870	1056	1094
195	284	512	694	793	879	1056	1105
195	287	512	695	793	881	1056	1106
195	289	512	696	793	882	1056	1108
195	291	512	697	793	883	1057	1077
195	295	512	698	793	885	1057	1080
195	296	512	699	793	890	1057	1083
195	297	512	700	793	891	1057	1084
195	305	512	701	793	892	1057	1085

FIG. 23

195	306	512	702	793	893	1057	1086
195	307	512	703	793	894	1057	1087
195	308	512	704	793	895	1057	1088
195	309	512	705	793	899	1057	1089
195	311	512	706	793	906	1057	1090
195	313	512	707	793	908	1057	1093
195	316	512	708	793	910	1057	1096
195	317	512	709	793	912	1057	1103
195	318	512	710	793	913	1057	1104
195	320	512	711	793	916	1057	1105
195	321	512	712	793	917	1057	1106
195	323	512	713	793	922	1058	1081
196	201	512	714	793	924	1058	1083
196	203	512	715	793	925	1058	1084
196	204	512	716	793	926	1058	1085
196	205	512	717	793	929	1058	1087
196	206	512	718	793	932	1058	1089
196	208	512	719	793	933	1058	1090
196	210	512	720	793	940	1058	1091
196	212	512	721	793	947	1058	1101
196	214	512	722	793	950	1058	1103
196	216	512	723	793	951	1058	1112
196	217	512	724	793	953	1059	1078
196	218	512	725	793	958	1059	1079
196	220	512	726	793	959	1059	1080

FIG. 23

196	222	512	728	793	960	1059	1081
196	223	512	729	793	961	1059	1082
196	224	512	730	793	968	1059	1083
196	226	512	731	793	977	1059	1084
196	227	512	732	794	798	1059	1085
196	228	512	734	794	799	1059	1086
196	229	513	654	794	810	1059	1087
196	230	513	658	794	823	1059	1088
196	231	513	686	794	826	1059	1089
196	232	513	713	794	843	1059	1091
196	233	514	590	794	856	1059	1094
196	234	514	647	794	868	1059	1106
196	237	514	648	794	880	1059	1107
196	239	514	650	794	881	1059	1111
196	240	514	652	794	898	1060	1080
196	241	514	653	794	901	1060	1081
196	242	514	654	794	916	1060	1083
196	243	514	661	794	917	1060	1084
196	244	514	668	794	918	1060	1085
196	245	514	675	794	923	1060	1086
196	246	514	676	794	924	1060	1087
196	247	514	679	794	925	1060	1089
196	248	514	685	794	933	1060	1090
196	250	514	686	794	950	1060	1091
196	252	514	698	794	951	1060	1094

FIG. 23

196	255	514	711	794	968	1060	1101
196	258	514	715	795	801	1060	1103
196	259	514	717	795	808	1060	1106
196	260	514	721	795	858	1062	1081
196	261	514	722	795	859	1062	1082
196	262	514	725	795	880	1062	1084
196	263	514	729	795	882	1062	1085
196	264	515	544	795	889	1062	1087
196	265	515	545	795	897	1062	1090
196	266	515	563	795	899	1062	1093
196	267	515	640	795	904	1062	1094
196	271	515	641	795	917	1062	1095
196	272	515	644	795	920	1062	1103
196	273	515	646	795	923	1062	1105
196	274	515	647	795	924	1063	1082
196	275	515	648	795	925	1063	1083
196	276	515	650	795	928	1063	1084
196	284	515	653	795	933	1063	1085
196	285	515	654	795	951	1063	1086
196	287	515	658	795	968	1063	1087
196	289	515	659	796	825	1063	1088
196	294	515	660	796	848	1063	1089
196	295	515	667	796	857	1063	1090
196	296	515	672	796	869	1063	1091
196	297	515	673	796	871	1063	1097

FIG. 23

196	300	515	686	796	881	1063	1102
196	301	515	687	796	898	1063	1104
196	305	515	688	796	900	1063	1107
196	306	515	689	796	901	1063	1110
196	308	515	690	796	902	1063	1111
196	311	515	698	796	903	1064	1083
196	312	515	701	796	904	1064	1086
196	313	515	704	796	908	1064	1105
196	315	515	708	796	909	1065	1084
196	316	515	710	796	911	1065	1085
196	317	515	712	796	915	1065	1087
196	318	515	715	796	916	1065	1088
196	319	515	716	796	917	1065	1089
196	321	515	717	796	919	1065	1090
196	323	515	724	796	920	1065	1105
196	324	515	725	796	923	1065	1110
197	201	515	728	796	924	1066	1085
197	205	515	729	796	925	1066	1086
197	211	516	635	796	927	1066	1087
197	213	516	643	796	932	1066	1088
197	217	516	644	796	933	1066	1089
197	218	516	646	796	937	1066	1090
197	224	516	647	796	942	1066	1105
197	226	516	648	797	814	1066	1106
197	227	516	650	797	826	1066	1109

FIG. 23

197	228	516	652	797	829	1066	1110
197	230	516	653	797	835	1067	1086
197	231	516	654	797	841	1067	1087
197	232	516	655	797	845	1067	1090
197	242	516	658	797	848	1067	1097
197	243	516	660	797	849	1067	1102
197	244	516	661	797	851	1067	1104
197	245	516	662	797	853	1067	1110
197	253	516	667	797	854	1067	1111
197	254	516	671	797	855	1067	1112
197	255	516	672	797	856	1068	1087
197	260	516	673	797	857	1068	1088
197	261	516	677	797	859	1068	1089
197	262	516	679	797	869	1068	1090
197	263	516	682	797	881	1068	1091
197	271	516	685	797	884	1068	1097
197	276	516	686	797	892	1068	1104
197	279	516	687	797	893	1068	1111
197	284	516	688	797	898	1069	1088
197	294	516	689	797	901	1069	1089
197	295	516	690	797	903	1069	1090
197	296	516	701	797	904	1069	1093
197	297	516	704	797	906	1069	1099
197	305	516	705	797	909	1069	1100
197	306	516	709	797	912	1069	1103

FIG. 23

197	314	516	710	797	916	1069	1107
197	316	516	712	797	917	1069	1110
197	318	516	714	797	920	1070	1095
197	319	516	715	797	921	1070	1096
197	321	516	717	797	923	1070	1108
198	203	516	721	797	924	1070	1109
198	208	516	723	797	925	1070	1110
198	211	516	724	797	928	1071	1090
198	212	516	728	797	929	1071	1101
198	214	516	729	797	932	1071	1104
198	217	517	548	797	933	1071	1107
198	218	517	571	797	937	1071	1110
198	220	517	606	797	942	1072	1091
198	223	517	614	797	948	1072	1094
198	226	517	626	797	949	1072	1107
198	228	517	638	797	951	1072	1108
198	230	517	642	797	956	1072	1111
198	231	517	644	797	968	1073	1103
198	232	517	645	798	802	1074	1093
198	234	517	646	798	805	1074	1094
198	240	517	647	798	806	1074	1095
198	243	517	649	798	810	1074	1108
198	244	517	650	798	822	1074	1111
198	245	517	652	798	823	1075	1095
198	247	517	653	798	824	1075	1101

FIG. 23

198	250	517	654	798	827	1075	1108
198	251	517	656	798	831	1076	1096
198	260	517	658	798	833	1076	1103
198	263	517	660	798	835	1076	1105
198	267	517	661	798	836	1076	1107
198	272	517	664	798	842	1076	1108
198	275	517	665	798	843	1076	1110
198	276	517	668	798	844	1077	1104
198	281	517	671	798	845	1077	1105
198	284	517	672	798	846	1077	1111
198	285	517	673	798	848	1078	1110
198	287	517	675	798	850	1079	1106
198	289	517	676	798	851	1080	1104
198	291	517	677	798	852	1082	1103
198	294	517	684	798	853	1082	1111
198	295	517	685	798	854	1082	1112
198	296	517	686	798	855	1083	1111
198	297	517	687	798	856	1084	1104
198	304	517	688	798	858	1085	1105
198	305	517	689	798	859	1085	1107
198	306	517	690	798	861	1085	1112
198	312	517	699	798	862	1086	1108
198	316	517	700	798	868	1088	1108
198	317	517	701	798	869	1088	1110
198	319	517	702	798	876	1088	1112

FIG. 23

198	321	517	703	798	877	1091	1110
198	324	517	704	798	878		
199	203	517	705	798	879		
199	204	517	707	798	881		
199	205	517	708	798	882		
199	211	517	709	798	883		
199	212	517	710	798	884		
199	215	517	712	798	885		
199	216	517	713	798	886		
199	217	517	714	798	888		
199	220	517	716	798	890		
199	222	517	717	798	891		
199	223	517	719	798	892		
199	224	517	721	798	893		
199	225	517	723	798	894		
199	227	517	724	798	897		
199	228	517	725	798	898		
199	229	517	728	798	899		
199	230	517	729	798	900		
199	231	517	730	798	901		
199	232	517	731	798	902		
199	233	518	626	798	903		
199	237	518	632	798	904		
199	242	518	633	798	905		
199	243	518	643	798	906		

FIG. 23

199	244	518	645	798	907
199	247	518	646	798	908
199	248	518	647	798	909
199	250	518	648	798	910
199	253	518	649	798	912
199	255	518	650	798	913
199	260	518	651	798	915
199	261	518	653	798	916
199	263	518	654	798	917
199	264	518	659	798	918
199	266	518	660	798	920
199	271	518	661	798	921
199	272	518	662	798	922
199	273	518	665	798	923
199	274	518	670	798	924
199	275	518	671	798	925
199	277	518	672	798	926
199	278	518	673	798	928
199	279	518	675	798	929
199	283	518	676	798	930
199	284	518	686	798	932
199	285	518	687	798	934
199	287	518	699	798	937
199	288	518	700	798	940
199	291	518	701	798	942

FIG. 23

199	294	518	702	798	945
199	295	518	704	798	947
199	296	518	705	798	948
199	297	518	706	798	950
199	300	518	709	798	951
199	301	518	711	798	954
199	302	518	712	798	957
199	304	518	713	798	958
199	305	518	716	798	959
199	306	518	717	798	960
199	312	518	722	798	961
199	313	518	725	798	975
199	314	518	728	798	979
199	316	518	729	798	980
199	318	518	730	798	981
199	319	518	739	799	803
199	321	519	552	799	812
200	205	519	572	799	832
200	211	519	642	799	836
200	216	519	644	799	848
200	218	519	645	799	850
200	221	519	647	799	851
200	222	519	650	799	852
200	226	519	652	799	853
200	227	519	654	799	855

FIG. 23

200	228	519	658	799	857
200	229	519	671	799	859
200	230	519	672	799	860
200	231	519	673	799	877
200	235	519	674	799	879
200	236	519	675	799	880
200	239	519	679	799	881
200	240	519	682	799	882
200	242	519	685	799	883
200	243	519	686	799	885
200	244	519	687	799	890
200	245	519	688	799	892
200	246	519	689	799	893
200	247	519	701	799	894
200	250	519	704	799	897
200	253	519	706	799	898
200	255	519	709	799	901
200	263	519	710	799	902
200	271	519	711	799	904
200	272	519	713	799	905
200	273	519	717	799	906
200	274	519	721	799	907
200	275	519	724	799	908
200	276	519	725	799	910
200	277	519	726	799	911

FIG. 23

200	278	519	730	799	912
200	279	520	572	799	915
200	280	520	606	799	916
200	283	520	625	799	917
200	287	520	642	799	920
200	289	520	647	799	921
200	290	520	648	799	922
200	295	520	649	799	923
200	296	520	653	799	924
200	297	520	658	799	925
200	301	520	660	799	929
200	305	520	663	799	930
200	306	520	673	799	932
200	309	520	675	799	933
200	314	520	677	799	937
200	317	520	687	799	938
200	318	520	688	799	941
200	319	520	698	799	947
201	205	520	703	799	948
201	211	520	704	799	950
201	214	520	713	799	954
201	216	520	716	799	961
201	217	520	717	799	968
201	220	520	721	799	971
201	225	520	725	799	979

FIG. 23

201	227	520	730	799	980
201	228	521	572	799	981
201	229	521	625	800	814
201	231	521	626	800	821
201	232	521	642	800	858
201	235	521	646	800	871
201	236	521	647	800	892
201	237	521	651	800	895
201	238	521	653	800	898
201	240	521	654	800	902
201	242	521	661	800	906
201	244	521	666	800	909
201	246	521	671	800	917
201	247	521	672	800	923
201	249	521	673	800	924
201	250	521	684	800	932
201	251	521	686	801	804
201	254	521	688	801	810
201	255	521	702	801	812
201	257	521	704	801	819
201	260	521	709	801	826
201	261	521	712	801	829
201	263	521	721	801	850
201	264	521	729	801	852
201	265	521	730	801	853

FIG. 23

201	266	522	556	801	854
201	271	522	562	801	858
201	272	522	573	801	870
201	274	522	583	801	880
201	275	522	585	801	881
201	279	522	590	801	882
201	281	522	598	801	883
201	283	522	610	801	884
201	284	522	620	801	885
201	285	522	621	801	890
201	287	522	622	801	891
201	289	522	628	801	895
201	291	522	630	801	898
201	294	522	631	801	899
201	295	522	632	801	901
201	296	522	635	801	902
201	297	522	636	801	905
201	301	522	637	801	906
201	305	522	640	801	908
201	306	522	641	801	909
201	308	522	642	801	911
201	309	522	643	801	912
201	314	522	644	801	915
201	315	522	645	801	916
201	317	522	646	801	918

FIG. 23

201	318	522	647	801	920
201	319	522	648	801	921
201	320	522	649	801	922
202	211	522	650	801	923
202	213	522	651	801	924
202	214	522	652	801	925
202	216	522	653	801	926
202	222	522	654	801	929
202	227	522	655	801	933
202	228	522	656	801	948
202	229	522	657	801	950
202	230	522	658	801	951
202	231	522	659	801	952
202	232	522	660	801	957
202	234	522	661	801	968
202	239	522	662	801	969
202	243	522	663	802	833
202	244	522	664	802	835
202	245	522	665	802	846
202	246	522	666	802	855
202	247	522	667	802	867
202	253	522	668	802	885
202	255	522	669	802	892
202	260	522	670	802	898
202	261	522	671	802	900

FIG. 23

202	264	522	672	802	908
202	271	522	673	802	909
202	272	522	674	802	910
202	274	522	675	802	915
202	276	522	677	802	917
202	277	522	678	802	918
202	279	522	679	802	920
202	283	522	680	802	921
202	284	522	681	802	922
202	287	522	682	802	924
202	289	522	683	802	927
202	294	522	684	802	929
202	295	522	686	802	932
202	297	522	687	802	947
202	306	522	688	802	949
202	312	522	689	803	817
202	314	522	690	803	859
202	315	522	691	803	881
202	316	522	692	803	892
202	317	522	693	803	897
202	319	522	694	803	901
202	324	522	695	803	925
203	211	522	696	803	933
203	223	522	697	803	951
203	227	522	698	804	810

FIG. 23

203	228	522	699	804	827
203	231	522	700	804	833
203	232	522	701	804	835
203	234	522	702	804	846
203	242	522	703	804	850
203	245	522	704	804	852
203	247	522	705	804	855
203	266	522	706	804	871
203	283	522	707	804	875
203	289	522	708	804	877
203	294	522	709	804	882
203	306	522	710	804	883
204	210	522	711	804	885
204	211	522	712	804	888
204	212	522	713	804	890
204	213	522	714	804	892
204	214	522	715	804	895
204	216	522	716	804	898
204	218	522	717	804	899
204	220	522	718	804	900
204	221	522	719	804	902
204	222	522	720	804	906
204	226	522	722	804	908
204	228	522	723	804	909
204	229	522	725	804	910

FIG. 23

204	230	522	726	804	913
204	231	522	727	804	918
204	232	522	730	804	920
204	233	523	554	804	921
204	237	523	562	804	922
204	242	523	577	804	923
204	243	523	581	804	927
204	244	523	585	804	929
204	247	523	586	804	937
204	250	523	589	804	938
204	255	523	590	804	940
204	259	523	592	805	831
204	261	523	594	805	898
204	265	523	595	805	910
204	271	523	598	805	911
204	272	523	600	805	915
204	274	523	601	805	916
204	276	523	604	805	922
204	279	523	606	805	924
204	281	523	607	806	920
204	289	523	609	806	933
204	290	523	620	806	971
204	291	523	621	807	810
204	294	523	623	807	813
204	295	523	624	807	824

FIG. 23

204	296	523	626	807	826
204	297	523	627	807	836
204	306	523	628	807	857
204	311	523	630	807	859
204	312	523	631	807	871
204	313	523	632	807	893
204	315	523	634	807	895
204	316	523	635	807	898
204	317	523	636	807	899
204	318	523	637	807	900
204	321	523	638	807	901
204	322	523	640	807	904
205	211	523	641	807	906
205	217	523	642	807	909
205	218	523	643	807	910
205	222	523	644	807	920
205	224	523	645	807	923
205	226	523	646	807	924
205	228	523	647	807	925
205	229	523	648	807	932
205	231	523	649	807	933
205	232	523	650	807	951
205	233	523	651	808	812
205	238	523	652	808	827
205	243	523	653	808	832

FIG. 23

205	244	523	654	808	835
205	245	523	655	808	843
205	246	523	656	808	844
205	248	523	658	808	852
205	249	523	659	808	864
205	250	523	660	808	868
205	251	523	661	808	881
205	254	523	662	808	883
205	255	523	663	808	885
205	258	523	664	808	899
205	260	523	665	808	902
205	261	523	666	808	903
205	264	523	667	808	904
205	265	523	668	808	908
205	266	523	669	808	911
205	272	523	670	808	912
205	273	523	671	808	917
205	275	523	672	808	918
205	281	523	673	808	921
205	284	523	674	808	923
205	285	523	675	808	924
205	287	523	676	808	925
205	289	523	677	808	926
205	291	523	678	808	932
205	294	523	679	808	950

FIG. 23

205	295	523	680	808	952
205	296	523	681	808	958
205	300	523	682	808	959
205	301	523	683	808	960
205	305	523	684	808	961
205	306	523	685	809	814
205	311	523	686	809	819
205	312	523	687	809	822
205	313	523	688	809	824
205	317	523	689	809	825
205	319	523	690	809	826
205	321	523	692	809	827
205	324	523	693	809	830
206	210	523	694	809	832
206	211	523	695	809	834
206	214	523	696	809	835
206	216	523	697	809	836
206	217	523	698	809	837
206	218	523	699	809	841
206	220	523	700	809	843
206	222	523	701	809	845
206	223	523	702	809	846
206	225	523	703	809	848
206	226	523	704	809	851
206	228	523	705	809	852

FIG. 23

206	229	523	706	809	853
206	231	523	707	809	854
206	232	523	708	809	855
206	233	523	709	809	856
206	234	523	710	809	857
206	235	523	711	809	860
206	236	523	712	809	866
206	239	523	713	809	868
206	240	523	714	809	869
206	241	523	715	809	870
206	242	523	716	809	874
206	243	523	717	809	876
206	244	523	718	809	877
206	246	523	719	809	878
206	247	523	720	809	879
206	248	523	721	809	882
206	249	523	722	809	883
206	250	523	723	809	884
206	251	523	725	809	885
206	252	523	726	809	886
206	253	523	728	809	888
206	254	523	729	809	890
206	255	523	730	809	892
206	256	523	732	809	893
206	259	523	737	809	897

FIG. 23

206	264	524	563	809	898
206	265	524	564	809	899
206	266	524	572	809	900
206	268	524	573	809	901
206	271	524	605	809	902
206	272	524	609	809	903
206	274	524	625	809	904
206	275	524	626	809	906
206	276	524	632	809	907
206	277	524	633	809	908
206	279	524	636	809	909
206	281	524	638	809	910
206	283	524	641	809	912
206	284	524	643	809	915
206	285	524	644	809	916
206	287	524	645	809	917
206	288	524	646	809	918
206	289	524	647	809	919
206	290	524	648	809	920
206	291	524	649	809	921
206	294	524	650	809	922
206	295	524	651	809	923
206	296	524	652	809	924
206	297	524	653	809	925
206	300	524	654	809	926

FIG. 23

206	303	524	658	809	927
206	305	524	659	809	928
206	306	524	660	809	929
206	307	524	661	809	931
206	308	524	662	809	932
206	312	524	664	809	933
206	313	524	665	809	938
206	315	524	667	809	941
206	316	524	668	809	942
206	317	524	669	809	950
206	318	524	670	809	951
206	319	524	671	809	954
206	321	524	672	809	955
207	212	524	673	809	957
207	217	524	674	809	958
207	223	524	675	809	959
207	227	524	676	809	961
207	228	524	677	809	963
207	231	524	678	809	968
207	233	524	679	809	976
207	235	524	680	809	977
207	237	524	681	809	978
207	242	524	682	810	830
207	243	524	683	810	848
207	245	524	684	810	853

FIG. 23

207	250	524	685	810	854
207	253	524	686	810	856
207	255	524	687	810	881
207	260	524	688	810	885
207	275	524	689	810	892
207	276	524	693	810	899
207	278	524	697	810	901
207	295	524	698	810	904
207	296	524	699	810	906
207	297	524	700	810	910
207	306	524	701	810	917
207	319	524	702	810	919
207	321	524	703	810	920
207	324	524	704	810	923
208	216	524	705	810	924
208	220	524	706	810	929
208	224	524	708	810	932
208	227	524	709	810	939
208	228	524	710	810	951
208	229	524	711	810	968
208	232	524	712	811	814
208	234	524	713	811	815
208	237	524	714	811	820
208	239	524	715	811	821
208	242	524	716	811	822

FIG. 23

208	243	524	717	811	826
208	244	524	720	811	827
208	246	524	721	811	830
208	248	524	722	811	831
208	250	524	724	811	832
208	255	524	725	811	837
208	262	525	562	811	840
208	264	525	568	811	844
208	272	525	572	811	847
208	276	525	580	811	848
208	281	525	585	811	850
208	284	525	595	811	851
208	285	525	597	811	853
208	287	525	598	811	854
208	289	525	602	811	855
208	291	525	603	811	860
208	296	525	607	811	862
208	297	525	613	811	867
208	305	525	617	811	868
208	306	525	620	811	869
208	316	525	621	811	870
208	320	525	626	811	871
208	321	525	628	811	873
209	213	525	631	811	875
209	232	525	634	811	876

FIG. 23

209	243	525	635	811	877
209	250	525	636	811	878
209	271	525	637	811	879
209	272	525	638	811	880
209	274	525	640	811	882
209	275	525	641	811	883
209	287	525	642	811	885
209	291	525	643	811	889
209	306	525	644	811	890
210	226	525	645	811	891
210	234	525	646	811	892
210	235	525	647	811	893
210	245	525	648	811	894
210	253	525	649	811	898
210	271	525	650	811	899
210	272	525	651	811	900
210	318	525	652	811	901
211	217	525	653	811	902
211	219	525	654	811	903
211	221	525	656	811	904
211	222	525	657	811	906
211	223	525	658	811	907
211	224	525	660	811	908
211	226	525	661	811	909
211	228	525	662	811	910

FIG. 23

211	230	525	663	811	912
211	232	525	664	811	913
211	233	525	665	811	915
211	234	525	666	811	916
211	235	525	667	811	917
211	239	525	668	811	918
211	240	525	669	811	920
211	242	525	670	811	921
211	243	525	671	811	922
211	244	525	672	811	923
211	250	525	673	811	924
211	255	525	674	811	925
211	260	525	675	811	926
211	272	525	677	811	927
211	275	525	678	811	928
211	276	525	679	811	929
211	278	525	680	811	937
211	279	525	681	811	942
211	283	525	682	811	948
211	284	525	683	811	950
211	287	525	684	811	957
211	291	525	685	811	958
211	293	525	686	811	959
211	294	525	687	811	961
211	295	525	688	811	964

FIG. 23

211	297	525	689	811	978
211	301	525	690	811	981
211	302	525	692	812	826
211	306	525	693	812	846
211	312	525	696	812	868
211	318	525	697	812	879
211	324	525	698	812	885
212	216	525	699	812	897
212	217	525	700	812	899
212	218	525	701	812	910
212	219	525	702	812	916
212	220	525	703	812	921
212	223	525	704	812	922
212	225	525	705	812	924
212	227	525	706	812	929
212	228	525	707	812	931
212	229	525	708	812	937
212	230	525	709	812	964
212	232	525	710	812	971
212	233	525	711	813	825
212	234	525	712	813	834
212	235	525	713	813	846
212	237	525	714	813	850
212	238	525	715	813	855
212	239	525	716	813	858

FIG. 23

212	240	525	717	813	869
212	243	525	719	813	881
212	244	525	720	813	884
212	245	525	722	813	885
212	246	525	723	813	890
212	247	525	725	813	898
212	248	525	726	813	899
212	250	525	730	813	900
212	251	526	644	813	901
212	253	526	646	813	904
212	254	526	647	813	906
212	255	526	652	813	908
212	256	526	653	813	910
212	257	526	654	813	912
212	258	526	655	813	913
212	259	526	660	813	917
212	260	526	661	813	918
212	262	526	665	813	920
212	263	526	667	813	921
212	264	526	673	813	923
212	265	526	674	813	924
212	266	526	678	813	925
212	269	526	680	813	929
212	271	526	682	813	932
212	272	526	684	813	956

FIG. 23

212	274	526	686	813	964
212	275	526	689	814	834
212	276	526	693	814	843
212	278	526	703	814	848
212	280	526	704	814	859
212	281	526	711	814	867
212	283	526	713	814	882
212	284	526	728	814	885
212	285	527	568	814	894
212	287	527	572	814	897
212	288	527	602	814	898
212	289	527	617	814	902
212	290	527	623	814	904
212	291	527	626	814	906
212	294	527	631	814	908
212	295	527	635	814	910
212	296	527	636	814	912
212	297	527	638	814	915
212	300	527	640	814	916
212	301	527	641	814	917
212	305	527	643	814	920
212	306	527	644	814	922
212	307	527	645	814	924
212	308	527	646	814	933
212	309	527	647	814	942

FIG. 23

212	311	527	648	814	951
212	314	527	649	814	958
212	315	527	650	814	977
212	316	527	651	815	820
212	317	527	652	815	823
212	318	527	654	815	826
212	319	527	656	815	827
212	320	527	658	815	832
212	321	527	659	815	835
212	322	527	660	815	837
212	323	527	661	815	843
212	324	527	662	815	845
213	218	527	664	815	846
213	220	527	665	815	848
213	222	527	667	815	850
213	226	527	668	815	851
213	227	527	669	815	854
213	228	527	670	815	856
213	229	527	671	815	857
213	232	527	672	815	858
213	233	527	673	815	859
213	234	527	674	815	863
213	235	527	675	815	865
213	236	527	676	815	867
213	239	527	677	815	870

FIG. 23

213	240	527	678	815	875
213	242	527	679	815	879
213	243	527	681	815	880
213	245	527	682	815	881
213	247	527	684	815	882
213	249	527	685	815	883
213	250	527	686	815	884
213	251	527	687	815	885
213	258	527	688	815	890
213	264	527	689	815	892
213	265	527	690	815	893
213	266	527	692	815	894
213	267	527	693	815	899
213	274	527	697	815	900
213	275	527	698	815	901
213	278	527	699	815	902
213	279	527	700	815	903
213	281	527	701	815	904
213	282	527	702	815	906
213	283	527	703	815	907
213	284	527	704	815	908
213	287	527	705	815	909
213	290	527	706	815	910
213	291	527	707	815	911
213	294	527	708	815	912

FIG. 23

213	295	527	710	815	915
213	297	527	712	815	916
213	302	527	713	815	917
213	305	527	714	815	918
213	306	527	715	815	919
213	311	527	716	815	920
213	312	527	717	815	921
213	316	527	718	815	923
213	318	527	719	815	924
213	319	527	720	815	925
213	320	527	721	815	926
214	218	527	722	815	927
214	220	527	723	815	928
214	222	527	725	815	929
214	223	527	727	815	932
214	224	527	728	815	933
214	226	527	729	815	950
214	227	527	730	815	951
214	228	528	562	815	978
214	231	528	624	815	981
214	232	528	626	816	848
214	233	528	642	816	880
214	234	528	644	816	892
214	236	528	645	816	894
214	239	528	647	816	919

FIG. 23

214	240	528	648	816	920
214	241	528	650	817	852
214	242	528	658	817	885
214	243	528	660	817	917
214	244	528	664	817	919
214	245	528	667	817	920
214	247	528	669	817	926
214	248	528	673	818	843
214	249	528	674	818	848
214	250	528	677	818	850
214	251	528	684	818	851
214	254	528	687	818	854
214	255	528	692	818	856
214	259	528	693	818	858
214	262	528	699	818	870
214	263	528	702	818	872
214	264	528	705	818	877
214	272	528	710	818	879
214	274	528	715	818	885
214	275	528	719	818	890
214	276	528	725	818	904
214	278	528	730	818	906
214	279	529	572	818	912
214	281	529	606	818	915
214	284	529	626	818	918

FIG. 23

214	285	529	632	818	920
214	286	529	641	818	921
214	289	529	643	818	923
214	290	529	646	818	924
214	291	529	647	818	926
214	293	529	648	818	929
214	294	529	649	818	930
214	296	529	654	818	932
214	297	529	660	818	933
214	301	529	662	818	934
214	304	529	665	819	857
214	306	529	669	819	867
214	307	529	671	819	868
214	308	529	672	819	880
214	309	529	673	819	898
214	313	529	684	819	902
214	315	529	687	819	904
214	316	529	697	819	909
214	317	529	698	819	912
214	319	529	701	819	914
214	320	529	702	819	916
214	321	529	704	819	917
215	222	529	705	819	919
215	224	529	707	819	924
215	226	529	708	819	926

FIG. 23

215	227	529	710	819	929
215	228	529	712	819	939
215	230	529	715	820	825
215	232	529	716	820	831
215	233	529	721	820	836
215	239	529	724	820	840
215	240	529	730	820	843
215	242	530	562	820	848
215	243	530	607	820	850
215	244	530	624	820	855
215	246	530	626	820	856
215	250	530	631	820	863
215	253	530	635	820	870
215	254	530	638	820	875
215	255	530	640	820	881
215	257	530	643	820	885
215	261	530	645	820	886
215	264	530	646	820	893
215	265	530	647	820	894
215	271	530	648	820	895
215	272	530	649	820	896
215	273	530	650	820	898
215	274	530	651	820	899
215	275	530	652	820	901
215	276	530	653	820	902

FIG. 23

215	279	530	654	820	903
215	283	530	656	820	904
215	284	530	657	820	905
215	287	530	658	820	906
215	289	530	660	820	908
215	291	530	661	820	909
215	294	530	662	820	912
215	295	530	663	820	913
215	296	530	664	820	916
215	297	530	665	820	917
215	299	530	667	820	918
215	301	530	668	820	920
215	304	530	669	820	921
215	305	530	671	820	923
215	306	530	672	820	924
215	307	530	673	820	925
215	311	530	674	820	929
215	312	530	675	820	932
215	317	530	677	820	933
215	318	530	679	820	942
215	319	530	680	820	971
215	321	530	681	820	973
216	220	530	683	820	978
216	222	530	684	820	980
216	223	530	685	820	981

FIG. 23

216	224	530	686	821	835
216	227	530	687	821	859
216	228	530	690	821	890
216	229	530	692	821	892
216	232	530	693	821	927
216	234	530	696	821	979
216	235	530	697	822	843
216	237	530	698	822	846
216	242	530	699	822	848
216	244	530	700	822	855
216	250	530	701	822	882
216	255	530	702	822	883
216	261	530	703	822	885
216	272	530	704	822	891
216	275	530	705	822	902
216	276	530	708	822	903
216	284	530	710	822	915
216	287	530	711	822	917
216	297	530	713	822	918
216	300	530	714	822	920
216	301	530	715	822	922
216	302	530	717	822	924
216	306	530	719	822	925
216	311	530	720	822	929
216	312	530	721	822	932

FIG. 23

216	317	530	722	822	979
216	318	530	725	822	980
216	321	530	730	823	830
217	222	531	603	823	831
217	223	531	638	823	834
217	224	531	642	823	840
217	225	531	643	823	843
217	226	531	644	823	848
217	227	531	652	823	850
217	228	531	653	823	853
217	231	531	654	823	854
217	232	531	655	823	855
217	233	531	658	823	860
217	235	531	659	823	866
217	239	531	660	823	871
217	240	531	661	823	876
217	241	531	663	823	877
217	242	531	665	823	880
217	243	531	667	823	881
217	244	531	672	823	882
217	245	531	673	823	885
217	246	531	674	823	891
217	247	531	675	823	893
217	248	531	686	823	895
217	249	531	687	823	899

FIG. 23

217	250	531	688	823	900
217	251	531	689	823	901
217	254	531	697	823	902
217	255	531	699	823	907
217	260	531	700	823	908
217	262	531	701	823	910
217	263	531	704	823	912
217	264	531	705	823	915
217	265	531	706	823	916
217	268	531	709	823	917
217	272	531	713	823	918
217	273	531	715	823	920
217	274	531	716	823	921
217	276	531	717	823	922
217	277	531	725	823	923
217	278	532	643	823	924
217	279	532	644	823	925
217	281	532	652	823	928
217	283	532	653	823	929
217	284	532	660	823	952
217	285	532	661	823	966
217	287	532	672	823	980
217	288	532	673	824	828
217	289	532	686	824	835
217	290	532	687	824	848

FIG. 23

217	291	532	698	824	854
217	293	532	705	824	855
217	294	532	711	824	857
217	295	532	716	824	868
217	296	533	604	824	870
217	297	533	647	824	871
217	301	533	648	824	876
217	305	533	660	824	878
217	306	533	661	824	879
217	308	533	671	824	881
217	311	533	672	824	882
217	312	533	673	824	883
217	314	533	686	824	884
217	315	533	697	824	885
217	316	533	699	824	888
217	317	533	700	824	890
217	318	533	702	824	891
217	319	533	705	824	892
217	321	533	712	824	893
217	322	533	716	824	894
217	323	533	717	824	895
217	324	534	568	824	896
218	222	534	572	824	897
218	223	534	590	824	898
218	224	534	621	824	899

FIG. 23

218	226	534	626	824	900
218	227	534	631	824	902
218	228	534	641	824	904
218	229	534	642	824	906
218	231	534	643	824	908
218	232	534	644	824	910
218	233	534	645	824	912
218	234	534	647	824	915
218	237	534	648	824	916
218	239	534	649	824	917
218	242	534	650	824	918
218	243	534	651	824	920
218	244	534	652	824	921
218	245	534	653	824	924
218	246	534	654	824	925
218	247	534	658	824	926
218	250	534	659	824	927
218	253	534	661	824	928
218	254	534	664	824	929
218	255	534	665	824	932
218	260	534	667	824	933
218	264	534	671	824	934
218	267	534	672	824	950
218	271	534	673	824	951
218	272	534	675	824	960

FIG. 23

218	274	534	676	824	964
218	276	534	679	824	966
218	284	534	680	824	969
218	285	534	683	824	975
218	287	534	685	824	979
218	290	534	686	825	828
218	291	534	687	825	830
218	294	534	688	825	831
218	295	534	689	825	832
218	296	534	690	825	835
218	297	534	697	825	836
218	305	534	698	825	839
218	306	534	699	825	845
218	307	534	701	825	848
218	312	534	702	825	850
218	314	534	703	825	852
218	317	534	704	825	853
218	318	534	705	825	854
218	319	534	706	825	855
218	321	534	708	825	856
218	324	534	709	825	857
219	226	534	710	825	858
219	230	534	711	825	862
219	234	534	712	825	868
219	235	534	713	825	871

FIG. 23

219	237	534	714	825	875
219	241	534	715	825	876
219	242	534	717	825	879
219	243	534	720	825	882
219	250	534	721	825	883
219	254	534	722	825	884
219	258	535	572	825	885
219	260	535	606	825	889
219	261	535	626	825	890
219	268	535	643	825	892
219	271	535	644	825	893
219	274	535	645	825	896
219	276	535	646	825	898
219	279	535	647	825	899
219	280	535	648	825	901
219	287	535	649	825	902
219	295	535	650	825	903
219	297	535	653	825	904
219	305	535	654	825	905
219	308	535	655	825	906
219	314	535	656	825	907
219	316	535	660	825	908
219	317	535	661	825	909
219	318	535	664	825	910
219	319	535	667	825	912

FIG. 23

219	321	535	669	825	915
220	242	535	671	825	916
220	244	535	672	825	917
220	250	535	673	825	918
220	253	535	675	825	919
220	254	535	676	825	920
220	287	535	684	825	921
220	297	535	685	825	922
220	301	535	686	825	923
220	312	535	687	825	924
220	314	535	688	825	925
221	226	535	689	825	927
221	227	535	697	825	929
221	228	535	699	825	932
221	229	535	701	825	933
221	230	535	702	825	937
221	231	535	703	825	938
221	233	535	704	825	950
221	234	535	705	825	956
221	235	535	707	825	960
221	236	535	708	825	972
221	240	535	709	825	977
221	242	535	710	825	981
221	243	535	712	826	831
221	244	535	713	826	849

FIG. 23

221	245	535	714	826	853
221	247	535	715	826	868
221	250	535	716	826	879
221	251	535	717	826	881
221	253	536	572	826	893
221	255	536	623	826	901
221	257	536	626	826	910
221	261	536	631	826	912
221	265	536	635	826	918
221	271	536	636	826	920
221	272	536	642	826	921
221	273	536	644	826	924
221	274	536	645	826	925
221	276	536	646	826	928
221	279	536	647	826	980
221	283	536	648	827	832
221	284	536	650	827	833
221	285	536	652	827	836
221	287	536	653	827	843
221	294	536	654	827	849
221	295	536	656	827	851
221	297	536	658	827	855
221	300	536	660	827	856
221	301	536	663	827	857
221	304	536	664	827	858

FIG. 23

221	306	536	665	827	862
221	307	536	668	827	866
221	314	536	671	827	870
221	316	536	672	827	871
221	317	536	673	827	874
221	318	536	674	827	876
221	323	536	675	827	879
222	227	536	678	827	881
222	229	536	680	827	882
222	230	536	682	827	883
222	239	536	683	827	885
222	242	536	684	827	886
222	243	536	686	827	890
222	246	536	687	827	891
222	250	536	688	827	892
222	253	536	689	827	893
222	254	536	697	827	897
222	274	536	698	827	898
222	275	536	699	827	899
222	296	536	701	827	900
222	301	536	704	827	901
222	305	536	705	827	904
222	324	536	706	827	906
223	228	536	708	827	907
223	230	536	710	827	908

FIG. 23

223	232	536	711	827	909
223	233	536	712	827	912
223	234	536	713	827	913
223	235	536	714	827	915
223	237	536	715	827	916
223	239	536	718	827	917
223	243	536	722	827	918
223	244	536	728	827	920
223	245	536	730	827	921
223	246	537	571	827	922
223	248	537	572	827	923
223	249	537	574	827	924
223	250	537	609	827	925
223	252	537	620	827	928
223	253	537	625	827	929
223	255	537	626	827	932
223	260	537	628	827	933
223	261	537	631	827	934
223	264	537	632	827	937
223	265	537	640	827	950
223	271	537	641	827	951
223	272	537	642	827	958
223	273	537	643	827	959
223	275	537	644	827	961
223	276	537	645	827	971

FIG. 23

223	279	537	646	827	975
223	281	537	647	827	980
223	283	537	648	828	835
223	284	537	649	828	842
223	285	537	650	828	843
223	287	537	651	828	844
223	289	537	652	828	848
223	295	537	653	828	849
223	296	537	654	828	850
223	299	537	655	828	855
223	302	537	658	828	856
223	306	537	660	828	857
223	308	537	661	828	858
223	311	537	662	828	859
223	312	537	663	828	864
223	317	537	664	828	869
223	319	537	665	828	871
223	320	537	666	828	880
223	323	537	667	828	883
223	324	537	668	828	895
224	228	537	669	828	898
224	229	537	670	828	900
224	230	537	671	828	901
224	232	537	672	828	902
224	235	537	673	828	906

FIG. 23

224	237	537	674	828	907
224	238	537	675	828	909
224	242	537	676	828	910
224	243	537	678	828	913
224	244	537	679	828	915
224	247	537	681	828	917
224	248	537	682	828	918
224	250	537	683	828	919
224	252	537	684	828	920
224	253	537	685	828	921
224	255	537	686	828	923
224	257	537	687	828	924
224	260	537	688	828	925
224	271	537	689	828	932
224	272	537	690	828	980
224	274	537	691	828	981
224	275	537	692	829	843
224	277	537	694	829	857
224	278	537	695	829	903
224	279	537	697	829	904
224	283	537	698	829	908
224	284	537	700	829	909
224	285	537	701	829	912
224	287	537	702	829	924
224	290	537	703	829	933

FIG. 23

224	291	537	704	829	979
224	297	537	705	829	980
224	300	537	706	830	835
224	302	537	707	830	838
224	305	537	708	830	840
224	311	537	709	830	856
224	314	537	710	830	860
224	319	537	711	830	869
224	321	537	712	830	871
225	230	537	713	830	881
225	232	537	714	830	882
225	233	537	715	830	885
225	235	537	716	830	898
225	236	537	717	830	899
225	237	537	719	830	900
225	242	537	720	830	902
225	244	537	721	830	903
225	247	537	722	830	906
225	250	537	723	830	908
225	251	537	727	830	910
225	255	537	729	830	920
225	258	537	730	830	921
225	260	537	733	830	922
225	263	538	570	830	923
225	264	538	572	830	924

FIG. 23

225	265	538	606	830	928
225	271	538	626	830	929
225	272	538	634	830	950
225	274	538	641	830	958
225	275	538	643	830	980
225	276	538	644	831	848
225	281	538	645	831	851
225	284	538	646	831	859
225	285	538	647	831	879
225	287	538	649	831	883
225	289	538	650	831	885
225	290	538	652	831	889
225	291	538	653	831	906
225	294	538	654	831	907
225	296	538	658	831	910
225	297	538	660	831	916
225	300	538	661	831	917
225	306	538	668	831	920
225	313	538	669	831	921
225	316	538	671	831	922
225	317	538	672	831	923
225	318	538	673	831	924
225	319	538	675	831	932
225	320	538	686	832	848
226	230	538	687	832	854

FIG. 23

226	231	538	697	832	856
226	250	538	698	832	868
226	253	538	701	832	882
226	261	538	702	832	890
226	297	538	703	832	899
226	301	538	704	832	900
226	314	538	705	832	901
226	318	538	708	832	904
227	231	538	709	832	916
227	232	538	711	832	918
227	233	538	712	832	920
227	239	538	715	832	923
227	240	538	716	832	924
227	242	538	717	832	925
227	243	538	723	832	956
227	244	538	730	832	979
227	247	539	571	833	844
227	248	539	577	833	848
227	250	539	631	833	853
227	254	539	633	833	857
227	255	539	646	833	858
227	261	539	647	833	867
227	262	539	648	833	871
227	267	539	652	833	877
227	271	539	654	833	880

FIG. 23

227	272	539	655	833	881
227	273	539	658	833	882
227	274	539	660	833	885
227	276	539	661	833	887
227	278	539	664	833	889
227	281	539	667	833	892
227	283	539	672	833	893
227	284	539	673	833	895
227	287	539	675	833	900
227	290	539	679	833	901
227	291	539	683	833	906
227	292	539	686	833	916
227	294	539	687	833	918
227	296	539	688	833	920
227	297	539	695	833	921
227	306	539	701	833	922
227	307	539	703	833	923
227	311	539	704	833	924
227	312	539	705	833	925
227	316	539	709	833	926
227	317	539	710	833	928
227	320	539	712	833	929
228	233	539	713	833	932
228	237	539	714	833	935
228	239	539	715	833	957

FIG. 23

228	242	539	718	833	960
228	243	539	722	833	979
228	244	539	723	833	980
228	245	540	649	833	981
228	246	540	655	834	848
228	250	540	660	834	854
228	253	540	672	834	873
228	255	540	673	834	880
228	257	540	683	834	881
228	261	540	686	834	892
228	271	540	688	834	898
228	272	540	697	834	900
228	273	540	702	834	901
228	274	540	703	834	902
228	275	540	706	834	904
228	277	540	711	834	906
228	279	540	713	834	908
228	283	540	716	834	909
228	284	540	717	834	910
228	287	541	574	834	911
228	292	541	609	834	912
228	296	541	641	834	916
228	297	541	642	834	917
228	300	541	644	834	919
228	302	541	647	834	920

FIG. 23

228	305	541	653	834	921
228	306	541	658	834	922
228	312	541	660	834	923
228	316	541	665	834	924
228	317	541	666	834	925
228	318	541	667	834	933
228	320	541	671	834	978
228	321	541	672	835	856
228	324	541	673	835	881
229	233	541	684	835	886
229	235	541	686	835	904
229	236	541	687	835	909
229	237	541	688	835	915
229	239	541	698	835	917
229	240	541	702	835	918
229	242	541	703	835	920
229	243	541	704	835	921
229	244	541	705	835	922
229	245	541	712	835	923
229	247	541	713	835	924
229	248	541	716	835	925
229	249	541	717	835	929
229	250	541	723	835	952
229	251	541	724	835	958
229	252	541	729	836	848

FIG. 23

229	253	541	731	836	851
229	254	542	673	836	852
229	255	542	675	836	853
229	258	542	686	836	854
229	260	542	710	836	856
229	263	542	715	836	857
229	264	542	717	836	860
229	272	543	647	836	866
229	273	543	654	836	869
229	274	543	671	836	871
229	275	543	683	836	873
229	276	543	686	836	876
229	277	543	709	836	877
229	279	543	730	836	880
229	284	544	604	836	881
229	285	544	606	836	883
229	287	544	625	836	885
229	289	544	626	836	886
229	290	544	632	836	888
229	291	544	633	836	891
229	294	544	641	836	892
229	295	544	643	836	893
229	296	544	644	836	894
229	297	544	645	836	895
229	300	544	646	836	898

FIG. 23

229	302	544	647	836	899
229	303	544	652	836	900
229	305	544	653	836	901
229	306	544	654	836	902
229	307	544	655	836	903
229	311	544	658	836	904
229	312	544	660	836	906
229	313	544	661	836	908
229	314	544	662	836	909
229	316	544	664	836	911
229	317	544	665	836	912
229	318	544	667	836	915
229	319	544	668	836	916
229	321	544	671	836	917
229	323	544	672	836	918
230	234	544	673	836	920
230	235	544	675	836	921
230	236	544	677	836	922
230	239	544	684	836	924
230	240	544	685	836	925
230	242	544	686	836	927
230	243	544	687	836	928
230	244	544	688	836	931
230	245	544	689	836	932
230	246	544	697	836	961

FIG. 23

230	247	544	698	836	969
230	248	544	699	836	978
230	249	544	701	837	848
230	250	544	702	837	849
230	251	544	703	837	853
230	252	544	704	837	857
230	253	544	705	837	858
230	254	544	706	837	860
230	257	544	708	837	880
230	258	544	709	837	883
230	260	544	710	837	885
230	261	544	711	837	888
230	262	544	712	837	893
230	264	544	713	837	894
230	265	544	716	837	900
230	266	544	717	837	906
230	271	544	721	837	908
230	272	544	725	837	911
230	274	544	734	837	916
230	276	545	593	837	917
230	278	545	600	837	919
230	279	545	606	837	920
230	281	545	625	837	924
230	283	545	626	837	932
230	289	545	641	837	933

FIG. 23

230	290	545	643	837	937
230	291	545	644	837	953
230	293	545	646	837	957
230	296	545	647	837	968
230	297	545	648	838	869
230	301	545	649	838	883
230	305	545	650	838	916
230	306	545	652	838	917
230	308	545	653	838	919
230	316	545	654	838	921
230	317	545	658	838	924
230	318	545	660	838	934
230	319	545	661	838	946
230	320	545	662	839	881
230	321	545	664	839	882
230	323	545	665	839	883
230	324	545	667	839	905
231	235	545	671	839	920
231	236	545	672	839	923
231	237	545	673	839	958
231	243	545	674	840	918
231	244	545	675	840	924
231	245	545	677	840	925
231	246	545	678	840	979
231	247	545	679	841	892

FIG. 23

231	248	545	681	841	900
231	249	545	682	841	909
231	250	545	683	841	925
231	252	545	684	841	956
231	257	545	685	842	846
231	264	545	686	842	855
231	265	545	687	842	869
231	268	545	688	842	881
231	274	545	692	842	882
231	278	545	697	842	885
231	283	545	698	842	892
231	288	545	699	842	900
231	291	545	701	842	901
231	294	545	702	842	905
231	295	545	703	842	906
231	296	545	704	842	908
231	297	545	705	842	912
231	300	545	706	842	917
231	301	545	708	842	918
231	305	545	709	842	919
231	306	545	710	842	921
231	308	545	711	842	922
231	313	545	712	843	848
231	316	545	713	843	850
231	317	545	715	843	853

FIG. 23

231	321	545	716	843	855
231	322	545	717	843	856
231	324	545	719	843	860
232	236	545	720	843	869
232	239	545	721	843	885
232	240	545	722	843	888
232	242	545	723	843	893
232	243	545	726	843	895
232	244	546	590	843	898
232	245	546	595	843	900
232	247	546	598	843	909
232	248	546	604	843	910
232	250	546	611	843	918
232	253	546	613	843	920
232	255	546	615	843	922
232	261	546	620	843	924
232	263	546	621	843	925
232	265	546	623	843	928
232	266	546	625	843	969
232	268	546	626	844	848
232	269	546	627	844	851
232	270	546	628	844	869
232	272	546	630	844	870
232	274	546	631	844	880
232	275	546	633	844	884

FIG. 23

232	278	546	634	844	902
232	281	546	635	844	904
232	284	546	636	844	906
232	285	546	638	844	907
232	287	546	639	844	908
232	289	546	640	844	912
232	290	546	641	844	913
232	291	546	642	844	916
232	292	546	643	844	920
232	294	546	644	844	921
232	295	546	645	844	922
232	296	546	646	844	923
232	297	546	647	844	924
232	300	546	648	844	925
232	304	546	649	844	929
232	306	546	650	844	933
232	309	546	651	844	971
232	311	546	652	844	979
232	312	546	653	845	851
232	315	546	654	845	857
232	316	546	656	845	901
232	317	546	657	845	904
232	318	546	658	845	909
232	322	546	659	845	910
233	239	546	660	845	916

FIG. 23

233	240	546	661	845	917
233	244	546	662	845	920
233	245	546	663	845	923
233	246	546	664	845	924
233	248	546	665	845	925
233	249	546	666	845	961
233	250	546	667	846	862
233	255	546	668	846	892
233	256	546	669	846	904
233	260	546	670	846	906
233	261	546	671	846	912
233	265	546	672	846	915
233	271	546	673	846	918
233	272	546	674	846	919
233	273	546	675	846	920
233	276	546	676	846	924
233	281	546	677	846	925
233	287	546	678	846	929
233	295	546	679	846	978
233	296	546	680	847	851
233	297	546	681	847	854
233	301	546	682	847	860
233	305	546	683	847	880
233	306	546	684	847	882
233	312	546	685	847	885

FIG. 23

233	317	546	686	847	895
233	318	546	687	847	901
233	321	546	688	847	902
234	239	546	689	847	904
234	241	546	690	847	909
234	242	546	691	847	920
234	243	546	692	847	924
234	244	546	693	847	925
234	247	546	694	847	979
234	250	546	695	848	857
234	253	546	696	848	885
234	255	546	697	848	895
234	260	546	698	848	898
234	261	546	699	848	916
234	263	546	700	848	917
234	265	546	701	848	920
234	271	546	702	848	924
234	272	546	703	848	925
234	274	546	704	849	852
234	283	546	705	849	853
234	284	546	706	849	855
234	285	546	707	849	857
234	287	546	708	849	860
234	291	546	709	849	867
234	294	546	710	849	876

FIG. 23

234	295	546	711	849	877
234	296	546	712	849	879
234	297	546	713	849	882
234	305	546	714	849	885
234	306	546	715	849	886
234	311	546	716	849	892
234	312	546	717	849	893
234	314	546	718	849	898
234	317	546	719	849	899
234	320	546	720	849	906
234	324	546	721	849	908
235	242	546	722	849	910
235	243	546	723	849	912
235	244	546	726	849	915
235	245	546	732	849	916
235	247	547	586	849	917
235	250	547	590	849	918
235	253	547	598	849	920
235	259	547	609	849	921
235	260	547	613	849	922
235	266	547	617	849	923
235	273	547	621	849	925
235	274	547	625	849	928
235	276	547	626	849	929
235	277	547	628	849	934

FIG. 23

235	284	547	629	849	950
235	285	547	630	849	951
235	287	547	631	849	961
235	290	547	634	849	967
235	297	547	635	849	981
235	305	547	636	850	869
235	306	547	638	850	870
235	316	547	640	850	875
236	240	547	641	850	877
236	244	547	642	850	883
236	245	547	643	850	894
236	248	547	644	850	901
236	251	547	645	850	906
236	253	547	646	850	913
236	255	547	647	850	915
236	257	547	648	850	920
236	258	547	649	850	921
236	263	547	650	850	959
236	264	547	651	850	981
236	265	547	652	851	855
236	268	547	653	851	860
236	271	547	654	851	894
236	274	547	656	851	899
236	275	547	658	851	901
236	276	547	659	851	902

FIG. 23

236	280	547	660	851	904
236	284	547	661	851	906
236	289	547	662	851	907
236	290	547	663	851	908
236	291	547	664	851	910
236	294	547	665	851	916
236	297	547	667	851	917
236	305	547	668	851	920
236	306	547	669	851	924
236	312	547	670	851	925
236	313	547	671	851	928
236	316	547	672	852	855
236	317	547	673	852	857
236	318	547	674	852	858
236	319	547	675	852	859
236	320	547	676	852	881
237	250	547	677	852	885
237	253	547	678	852	890
237	264	547	679	852	892
237	272	547	680	852	895
237	273	547	682	852	898
237	276	547	683	852	899
237	287	547	684	852	900
237	305	547	685	852	901
237	314	547	686	852	902

FIG. 23

237	316	547	687	852	904
237	318	547	688	852	908
238	242	547	690	852	909
238	244	547	693	852	910
238	245	547	695	852	912
238	250	547	696	852	917
238	264	547	697	852	918
238	271	547	698	852	919
238	272	547	699	852	920
238	273	547	700	852	921
238	274	547	701	852	923
238	275	547	702	852	924
238	277	547	703	852	925
238	278	547	704	852	928
238	284	547	705	852	929
238	287	547	706	852	933
238	291	547	708	852	971
238	295	547	710	852	981
238	305	547	711	853	858
238	306	547	712	853	880
239	243	547	713	853	882
239	244	547	714	853	885
239	245	547	715	853	886
239	250	547	716	853	892
239	273	547	717	853	893

FIG. 23

239	275	547	718	853	895
239	294	547	719	853	897
239	296	547	720	853	898
239	297	547	721	853	901
239	305	547	722	853	904
239	319	547	723	853	908
240	244	547	725	853	923
240	245	547	731	853	924
240	247	547	732	853	925
240	249	547	735	853	928
240	250	548	577	853	934
240	253	548	590	853	981
240	260	548	621	854	859
240	261	548	628	854	869
240	271	548	630	854	880
240	272	548	631	854	881
240	273	548	632	854	917
240	274	548	637	854	924
240	276	548	641	855	867
240	278	548	642	855	871
240	281	548	643	855	881
240	291	548	645	855	883
240	295	548	646	855	888
240	296	548	647	855	890
240	297	548	648	855	895

FIG. 23

240	300	548	649	855	898
240	305	548	650	855	905
240	317	548	651	855	906
240	318	548	652	855	907
240	321	548	654	855	910
241	245	548	656	855	915
241	247	548	658	855	916
241	250	548	659	855	917
241	253	548	660	855	918
241	254	548	662	855	920
241	260	548	663	855	922
241	263	548	664	855	923
241	267	548	665	855	924
241	272	548	669	855	925
241	275	548	671	855	980
241	276	548	672	856	869
241	281	548	673	856	870
241	287	548	674	856	878
241	289	548	675	856	879
241	294	548	677	856	880
241	295	548	678	856	881
241	302	548	679	856	882
241	311	548	680	856	885
241	312	548	681	856	888
241	316	548	682	856	898

FIG. 23

241	322	548	683	856	899
242	250	548	684	856	900
242	273	548	687	856	902
242	276	548	688	856	903
242	287	548	689	856	904
242	297	548	692	856	907
243	247	548	693	856	909
243	250	548	694	856	910
243	251	548	696	856	915
243	254	548	697	856	916
243	257	548	699	856	918
243	263	548	700	856	919
243	264	548	701	856	920
243	265	548	702	856	921
243	268	548	703	856	922
243	274	548	704	856	923
243	275	548	705	856	924
243	277	548	707	856	925
243	278	548	708	856	927
243	279	548	711	856	949
243	281	548	712	856	961
243	284	548	713	856	968
243	285	548	714	856	971
243	287	548	715	856	978
243	289	548	716	856	980

FIG. 23

243	290	548	720	856	981
243	291	548	721	857	870
243	294	548	722	857	871
243	295	548	723	857	872
243	296	548	726	857	876
243	297	548	737	857	879
243	305	548	739	857	882
243	306	549	610	857	883
243	307	549	623	857	884
243	312	549	626	857	885
243	313	549	627	857	892
243	317	549	628	857	893
243	320	549	631	857	895
243	321	549	634	857	896
243	323	549	635	857	898
243	324	549	636	857	899
244	248	549	641	857	901
244	250	549	644	857	902
244	251	549	645	857	904
244	253	549	646	857	905
244	254	549	647	857	906
244	255	549	648	857	908
244	257	549	649	857	910
244	260	549	650	857	915
244	261	549	652	857	916

FIG. 23

244	263	549	653	857	918
244	264	549	654	857	920
244	265	549	658	857	921
244	266	549	659	857	922
244	267	549	660	857	923
244	271	549	661	857	924
244	272	549	662	857	925
244	273	549	663	857	926
244	274	549	664	857	929
244	275	549	665	857	938
244	277	549	666	857	951
244	278	549	667	857	957
244	280	549	668	857	961
244	281	549	669	858	869
244	283	549	670	858	870
244	284	549	671	858	873
244	285	549	672	858	876
244	287	549	673	858	878
244	291	549	674	858	879
244	295	549	675	858	880
244	297	549	677	858	881
244	301	549	678	858	882
244	302	549	679	858	883
244	304	549	680	858	885
244	306	549	682	858	887

FIG. 23

244	312	549	683	858	890
244	314	549	684	858	892
244	316	549	685	858	895
244	317	549	686	858	899
244	318	549	687	858	900
244	319	549	688	858	901
244	320	549	689	858	906
244	324	549	690	858	907
245	250	549	692	858	910
245	253	549	693	858	915
245	254	549	695	858	917
245	255	549	697	858	920
245	257	549	698	858	921
245	259	549	699	858	922
245	260	549	700	858	923
245	261	549	701	858	924
245	264	549	702	858	925
245	265	549	703	858	926
245	266	549	704	858	928
245	267	549	705	858	931
245	268	549	706	858	937
245	271	549	708	858	950
245	272	549	709	858	951
245	273	549	710	858	957
245	274	549	711	858	968

FIG. 23

245	275	549	712	859	869
245	276	549	713	859	882
245	277	549	714	859	894
245	279	549	715	859	901
245	281	549	717	859	906
245	283	549	718	859	917
245	284	549	719	859	920
245	287	549	720	859	924
245	294	549	721	859	925
245	295	549	722	859	927
245	296	549	723	859	932
245	297	549	724	859	941
245	301	549	725	859	963
245	302	549	726	859	971
245	306	549	736	860	876
245	311	550	645	860	890
245	312	550	647	860	892
245	314	550	649	860	904
245	315	550	653	860	906
245	316	550	654	860	908
245	317	550	660	860	912
245	318	550	661	860	915
245	319	550	671	860	917
245	322	550	672	860	918
245	323	550	686	860	920

FIG. 23

246	250	550	687	860	924
246	252	550	704	860	925
246	253	550	708	860	926
246	255	550	713	860	927
246	260	550	717	860	928
246	261	550	730	860	958
246	264	551	597	860	964
246	271	551	600	860	970
246	272	551	625	860	973
246	273	551	641	860	980
246	274	551	643	861	880
246	275	551	644	861	883
246	276	551	645	861	892
246	283	551	646	861	895
246	284	551	647	861	901
246	287	551	649	861	906
246	291	551	653	861	909
246	294	551	654	861	924
246	295	551	660	861	925
246	297	551	661	862	901
246	304	551	667	862	926
246	305	551	672	863	909
246	306	551	673	863	910
246	312	551	675	863	919
246	314	551	683	863	920

FIG. 23

246	317	551	684	863	925
246	324	551	688	864	881
247	255	551	689	864	892
247	272	551	695	864	898
247	274	551	697	864	902
247	279	551	699	864	904
247	282	551	703	864	921
247	283	551	704	864	937
247	297	551	705	865	975
247	301	551	706	867	902
247	313	551	707	867	909
247	315	551	708	867	916
247	320	551	709	867	924
247	324	551	710	867	951
248	271	551	712	867	980
248	272	551	713	868	876
248	273	551	715	868	901
248	275	551	716	868	906
248	276	551	718	868	908
248	277	551	721	868	916
248	278	551	722	868	923
248	280	551	723	868	925
248	297	551	725	868	955
248	301	552	581	868	978
248	306	552	626	869	881

FIG. 23

248	312	552	635	869	882
248	318	552	638	869	883
248	324	552	640	869	902
249	253	552	646	869	904
249	255	552	647	869	921
249	260	552	654	869	924
249	261	552	658	869	926
249	273	552	660	869	928
249	275	552	661	869	929
249	276	552	664	869	977
249	283	552	669	870	879
249	287	552	671	870	918
249	300	552	672	870	922
249	302	552	673	871	901
249	304	552	675	871	910
249	306	552	676	871	915
249	316	552	678	871	919
249	319	552	679	871	920
250	255	552	680	871	924
250	271	552	683	871	925
250	272	552	686	871	973
250	287	552	688	871	981
250	318	552	698	872	881
250	321	552	700	872	898
251	258	552	701	872	901

FIG. 23

251	260	552	702	872	906
251	262	552	704	872	909
251	263	552	705	872	910
251	264	552	706	872	918
251	266	552	708	872	923
251	267	552	709	872	924
251	274	552	711	872	925
251	276	552	712	872	928
251	277	552	713	872	971
251	278	552	714	873	892
251	279	552	716	873	917
251	280	552	717	873	980
251	283	552	718	874	883
251	284	552	721	874	893
251	287	552	723	874	916
251	289	552	725	874	918
251	291	552	726	874	923
251	292	552	727	874	925
251	294	552	730	874	941
251	296	552	734	874	953
251	305	553	609	875	901
251	306	553	626	876	879
251	307	553	644	876	882
251	313	553	645	876	892
251	316	553	647	876	904

FIG. 23

251	317	553	652	876	906
251	319	553	654	876	922
251	321	553	658	876	924
252	261	553	661	876	951
252	272	553	664	877	889
252	283	553	667	877	908
252	296	553	673	877	916
252	305	553	675	877	917
252	312	553	686	877	924
252	316	553	687	878	901
253	319	553	689	878	908
254	258	553	697	878	909
254	264	553	700	878	924
254	265	553	704	879	907
254	276	553	707	879	920
254	283	553	708	879	925
254	287	553	709	879	934
254	291	553	711	879	941
254	296	553	712	879	980
254	306	553	713	880	892
254	307	553	715	880	901
254	308	553	717	880	917
254	309	553	723	880	919
254	317	554	647	880	921
254	318	554	658	880	922

FIG. 23

254	323	554	673	880	924
255	270	554	702	880	925
255	279	554	704	880	941
255	287	554	705	880	980
255	306	554	708	881	891
255	319	554	710	881	894
256	260	554	712	881	899
256	271	555	609	881	900
256	272	555	644	881	902
256	273	555	645	881	904
256	274	555	646	881	910
256	279	555	654	881	916
256	283	555	658	881	917
256	284	555	673	881	922
256	287	555	674	881	926
256	290	555	675	881	928
256	306	555	685	881	929
256	311	555	686	881	942
256	312	555	688	881	969
256	316	555	697	881	971
256	322	555	702	881	980
258	274	555	704	882	894
258	284	555	708	882	899
258	291	555	709	882	900
259	316	555	711	882	903

FIG. 23

260	274	555	713	882	904
260	291	555	715	882	910
260	323	555	716	882	916
261	265	555	717	882	917
261	274	555	730	882	920
261	284	556	625	882	922
261	291	556	643	882	924
261	294	556	644	882	929
261	317	556	645	882	932
262	268	556	647	882	950
262	274	556	651	882	961
262	281	556	652	883	901
262	284	556	654	883	902
262	291	556	655	883	904
262	294	556	658	883	906
262	306	556	660	883	920
262	316	556	672	883	923
262	317	556	686	883	925
264	295	556	687	883	933
264	306	556	688	883	941
265	274	556	697	883	980
265	275	556	699	884	893
265	276	556	704	884	902
265	287	556	706	884	904
265	312	556	713	884	916

FIG. 23

265	317	556	716	884	919
266	294	556	717	884	924
268	305	556	729	884	929
272	296	557	609	884	933
273	285	557	626	884	981
273	289	557	647	885	892
273	291	557	654	885	901
273	299	557	661	885	904
273	300	557	673	885	924
273	315	557	686	885	925
274	281	557	697	886	901
274	289	557	701	886	904
274	291	557	704	886	906
275	283	557	705	886	908
275	287	557	708	886	913
275	290	557	716	886	915
275	294	557	717	886	917
276	290	558	641	886	919
276	291	558	660	886	920
276	295	558	683	886	924
276	313	559	625	886	925
276	315	559	643	886	941
276	318	559	644	886	951
277	285	559	646	886	979
277	291	559	652	886	980

FIG. 23

277	305	559	653	887	901
277	317	559	654	887	920
278	296	559	671	887	925
279	289	559	673	887	980
279	294	559	677	889	925
279	315	559	680	890	909
279	319	559	681	890	920
280	284	559	683	890	949
280	285	559	687	891	898
280	287	559	688	891	916
280	296	559	702	891	920
280	311	559	705	891	923
280	319	559	706	891	924
281	315	559	710	891	925
282	289	559	711	892	895
282	305	559	713	892	898
282	306	559	717	892	904
283	287	559	723	892	907
284	289	560	641	892	924
284	291	560	643	893	898
284	300	560	645	893	907
284	323	560	647	893	911
285	289	560	649	893	916
285	292	560	653	893	919
285	296	560	654	893	920

FIG. 23

285	297	560	658	893	921
285	299	560	671	893	923
285	305	560	672	893	924
287	323	560	673	893	929
288	292	560	675	893	950
288	294	560	683	893	961
288	296	560	687	893	980
288	306	560	702	894	904
288	310	560	704	894	907
288	318	560	705	894	908
288	319	560	709	894	910
288	320	560	715	894	916
289	318	560	716	894	917
290	305	560	717	894	919
290	312	560	732	894	920
290	319	561	719	894	923
292	297	561	720	894	924
292	319	562	631	894	925
296	317	562	645	894	927
298	306	562	647	894	934
298	315	562	648	894	968
298	319	562	649	894	978
298	320	562	652	894	981
300	306	562	654	895	898
301	306	562	660	895	909

FIG. 23

301	307	562	672	895	913
303	310	562	686	895	917
306	310	562	689	895	925
307	316	562	697	895	979
307	317	562	700	896	908
307	322	562	701	896	919
307	323	562	702	896	924
313	321	562	704	896	951
313	323	562	708	896	971
		562	712	897	917
		562	713	897	951
		562	717	897	977
		562	718	897	980
		563	609	898	904
		563	623	898	917
		563	625	898	925
		563	626	899	906
		563	633	899	908
		563	641	899	912
		563	643	899	916
		563	644	899	919
		563	645	899	920
		563	646	899	921
		563	647	899	923
		563	649	899	925

FIG. 23

563	650	899	926
563	651	899	929
563	652	899	951
563	653	899	971
563	654	900	910
563	658	900	920
563	660	900	924
563	661	900	925
563	662	901	910
563	663	901	921
563	668	901	922
563	669	901	958
563	670	901	964
563	671	902	908
563	672	902	910
563	673	902	913
563	675	902	918
563	676	902	921
563	679	902	922
563	682	902	924
563	683	902	925
563	684	902	927
563	685	902	929
563	686	902	953
563	687	902	973

FIG. 23

563	688	903	909
563	690	903	916
563	697	903	924
563	698	903	925
563	699	903	937
563	700	903	951
563	701	903	966
563	702	903	978
563	703	904	980
563	704	905	908
563	705	905	920
563	706	905	921
563	708	905	922
563	709	905	923
563	710	905	925
563	711	905	927
563	712	905	941
563	713	905	973
563	714	907	915
563	715	907	916
563	716	907	918
563	717	907	919
563	729	907	920
563	730	907	925
563	737	907	929

FIG. 23

564	598	907	942
564	633	907	979
564	635	908	950
564	638	909	912
564	640	909	915
564	642	909	916
564	644	909	920
564	645	909	921
564	646	909	925
564	647	909	929
564	648	909	932
564	649	909	937
564	651	909	968
564	653	909	977
564	654	910	921
564	656	910	925
564	658	910	980
564	659	911	916
564	660	911	920
564	662	911	924
564	663	911	929
564	664	911	958
564	665	911	971
564	669	911	979
564	670	912	929

FIG. 23

564	671	912	957
564	673	913	924
564	674	913	931
564	675	914	917
564	678	914	920
564	679	914	925
564	682	914	929
564	683	916	921
564	684	916	923
564	686	916	925
564	688	916	927
564	689	916	963
564	692	916	980
564	697	917	920
564	698	917	921
564	701	917	928
564	702	917	934
564	703	917	957
564	704	918	924
564	705	918	925
564	706	918	933
564	708	918	950
564	710	918	980
564	711	918	981
564	712	919	924

FIG. 23

564	713	919	925
564	714	919	933
564	716	921	924
564	717	921	925
564	719	921	928
564	720	921	929
564	736	921	933
565	626	922	928
565	634	922	929
565	643	922	933
565	654	922	956
565	660	922	961
565	686	922	978
565	688	922	980
565	699	922	981
565	704	924	928
565	705	924	958
565	713	925	929
565	714	925	932
565	717	925	942
565	719	925	956
566	596	925	961
566	626	925	963
566	635	926	964
566	636	926	972

FIG. 23

566	647	926	978
566	648	930	955
566	649	933	980
566	650	934	981
566	652	938	951
566	654	938	962
566	661	939	950
566	663	939	973
566	667	940	953
566	670	940	971
566	672	942	952
566	674	943	950
566	675	943	981
566	676	944	951
566	678	945	948
566	679	949	980
566	682	955	981
566	683	957	965
566	684	958	981
566	687	959	980
566	690	960	964
566	692	962	980
566	696	969	975
566	701	970	979
566	702	971	981

FIG. 23

566	705	972	975
566	706	972	977
566	708	972	978
566	710	972	979
566	711	972	980
566	713	972	981
566	714	974	980
566	715	975	980
566	717	976	980
566	719	977	981
566	721	978	981
566	739		
567	638		
567	647		
567	673		
567	678		
567	697		
567	718		
568	640		
568	643		
568	645		
568	646		
568	651		
568	654		
568	660		

FIG. 23

568	666
568	672
568	673
568	677
568	680
568	688
568	697
568	698
568	702
568	703
568	704
568	710
568	713
568	714
568	719
569	606
569	644
569	646
569	647
569	652
569	654
569	656
569	659
569	660
569	661

FIG. 23

569	663
569	667
569	671
569	672
569	673
569	675
569	682
569	684
569	686
569	688
569	697
569	698
569	700
569	702
569	703
569	706
569	710
569	714
569	723
570	631
570	650
570	673
570	703
571	647
572	637

FIG. 23

572	643
572	644
572	645
572	646
572	647
572	648
572	654
572	658
572	660
572	661
572	662
572	663
572	672
572	673
572	674
572	675
572	677
572	682
572	683
572	684
572	686
572	687
572	688
572	689
572	692

FIG. 23

572	697
572	703
572	704
572	705
572	707
572	708
572	710
572	711
572	713
572	715
572	717
572	722
572	729
572	730
573	626
573	628
573	630
573	631
573	635
573	636
573	643
573	645
573	646
573	647
573	648

FIG. 23

573	649
573	650
573	651
573	652
573	653
573	656
573	657
573	658
573	659
573	660
573	661
573	662
573	663
573	664
573	665
573	666
573	667
573	669
573	670
573	671
573	672
573	673
573	674
573	675
573	677

FIG. 23

573	679
573	680
573	682
573	683
573	684
573	685
573	686
573	687
573	688
573	692
573	695
573	697
573	699
573	701
573	702
573	703
573	704
573	705
573	706
573	708
573	709
573	710
573	713
573	714
573	715

FIG. 23

573	716
573	717
573	719
573	726
573	729
573	739
574	604
574	606
574	613
574	623
574	624
574	631
574	640
574	641
574	646
574	647
574	648
574	649
574	650
574	652
574	653
574	654
574	655
574	660
574	661

FIG. 23

574	662
574	664
574	666
574	669
574	670
574	671
574	672
574	673
574	674
574	675
574	676
574	677
574	678
574	679
574	683
574	684
574	686
574	687
574	688
574	689
574	693
574	695
574	697
574	699
574	700

FIG. 23

574	701
574	702
574	703
574	705
574	706
574	708
574	710
574	711
574	712
574	713
574	714
574	715
574	717
574	719
574	722
574	723
574	732
575	607
575	613
575	635
575	638
575	640
575	642
575	645
575	648

FIG. 23

575	649
575	650
575	652
575	653
575	658
575	662
575	664
575	666
575	668
575	670
575	671
575	672
575	673
575	674
575	677
575	678
575	679
575	682
575	683
575	684
575	686
575	687
575	689
575	691
575	699

FIG. 23

575	701
575	702
575	703
575	705
575	706
575	709
575	714
575	715
575	719
575	720
575	722
575	739
576	626
576	637
576	647
576	650
576	654
576	658
576	670
576	673
576	677
576	686
576	692
576	701
576	702

FIG. 23

576	704
576	706
576	710
576	713
576	714
577	626
577	661
577	664
577	665
577	673
577	687
577	705
577	710
577	717
577	729
578	660
578	699
578	721
579	646
579	661
579	665
579	686
579	688
579	716
580	626

FIG. 23

580	716
581	644
581	645
581	646
581	649
581	654
581	660
581	673
581	687
581	688
581	704
581	706
581	710
581	711
581	712
581	717
582	648
582	673
582	686
582	688
582	689
582	705
584	688
585	651
585	671

FIG. 23

585	716
586	654
586	660
586	673
586	675
586	688
586	704
586	705
586	709
587	644
587	653
587	654
587	685
587	704
587	707
587	712
587	716
588	641
588	644
588	646
588	650
588	658
588	660
588	662
588	668

FIG. 23

588	672
588	675
588	686
588	688
588	697
588	702
588	707
588	708
588	709
588	713
588	715
588	717
589	654
589	661
590	628
590	641
590	643
590	644
590	647
590	649
590	650
590	654
590	658
590	661
590	668

FIG. 23

590	671
590	673
590	686
590	687
590	688
590	698
590	699
590	700
590	702
590	703
590	704
590	712
590	713
590	716
590	717
590	723
590	729
591	641
591	646
591	647
591	649
591	654
591	660
591	661
591	668

FIG. 23

591	671
591	672
591	685
591	686
591	687
591	688
591	691
591	697
591	700
591	701
591	702
591	704
591	706
591	709
591	711
591	712
591	713
591	715
591	716
591	717
591	720
592	665
592	673
592	686
593	654

FIG. 23

593	704
594	644
594	673
594	697
594	702
594	710
594	712
594	715
594	717
594	719
595	641
595	647
595	653
595	654
595	658
595	665
595	666
595	673
595	675
595	685
595	697
595	702
595	705
595	709
595	711

FIG. 23

595	714
595	715
595	724
595	730
596	644
596	646
596	653
596	673
596	705
596	706
596	712
596	717
596	724
597	626
597	644
597	647
597	654
597	687
597	688
597	702
597	709
597	713
597	729
598	672
600	645

FIG. 23

600	660
600	673
600	675
600	713
601	643
601	646
601	647
601	661
601	671
601	672
601	673
601	687
601	705
601	712
601	713
602	649
602	658
602	673
602	697
602	705
602	709
602	738
603	645
603	647
603	661

FIG. 23

603	673
603	702
603	704
603	705
603	709
603	713
603	730
604	650
604	673
604	678
604	680
604	683
604	684
604	687
604	696
604	705
604	710
604	712
605	646
605	647
605	652
605	654
605	668
605	672
605	680

FIG. 23

605	687
605	698
605	704
605	708
605	709
605	710
605	729
606	702
606	704
606	705
606	710
607	648
607	650
607	658
607	661
607	663
607	665
607	667
607	669
607	672
607	673
607	676
607	677
607	680
607	682

FIG. 23

607	684
607	686
607	690
607	692
607	693
607	694
607	701
607	702
607	703
607	704
607	705
607	706
607	708
607	710
607	711
607	713
607	714
607	715
607	717
607	719
607	730
607	739
608	649
608	654
608	659

FIG. 23

608	661
608	671
608	687
608	702
608	703
608	704
608	712
608	717
610	643
610	647
610	650
610	651
610	654
610	655
610	658
610	659
610	661
610	664
610	667
610	669
610	672
610	673
610	674
610	679
610	680

FIG. 23

610	682
610	684
610	687
610	689
610	704
610	705
610	706
610	708
610	709
610	711
610	713
610	714
610	715
610	723
610	728
611	641
611	644
611	645
611	647
611	654
611	660
611	688
611	699
611	702
611	706

FIG. 23

611	716
611	717
612	644
612	646
612	660
612	716
614	646
614	658
614	673
614	676
614	687
614	688
614	689
614	704
614	705
614	707
614	710
614	716
614	723
614	738
615	647
615	705
618	654
618	660
618	706

FIG. 23

618	713
618	738
619	715
620	712
621	671
621	687
621	702
621	704
621	705
621	716
621	729
622	660
622	665
622	672
622	673
622	675
622	680
622	685
622	686
622	687
622	688
622	704
622	705
622	708
622	709

FIG. 23

622	712
622	717
622	729
622	731
622	732
623	654
623	686
623	699
624	660
624	696
624	701
624	715
624	729
625	673
625	692
625	697
625	715
625	723
625	728
626	658
626	661
626	663
626	669
626	673
626	674

FIG. 23

626	680
626	681
626	682
626	684
626	701
626	703
626	704
626	710
626	714
626	726
626	738
627	656
627	658
627	660
627	661
627	673
627	675
627	677
627	685
627	686
627	688
627	698
627	701
627	704
627	705

FIG. 23

627	706
627	713
628	675
628	704
629	660
629	671
629	673
629	683
629	688
629	697
629	698
629	703
629	704
629	705
629	709
629	710
629	711
629	712
629	713
629	714
629	716
629	723
629	732
630	664
630	665

FIG. 23

630	673
630	691
630	704
630	711
630	714
631	672
631	673
631	674
631	675
631	686
631	697
631	698
631	702
631	703
631	704
631	705
631	712
631	715
631	717
632	661
632	667
632	668
632	671
632	672
632	673

FIG. 23

632	674
632	677
632	685
632	686
632	687
632	688
632	689
632	700
632	706
632	709
632	710
632	712
632	713
632	715
632	717
632	727
632	729
632	738
633	662
633	697
634	664
634	677
634	679
634	680
634	681

FIG. 23

634	683
634	684
634	687
634	692
634	693
634	697
634	698
634	699
634	702
634	703
634	708
634	710
634	711
634	712
634	713
634	714
634	715
634	725
634	730
635	670
635	686
635	687
635	689
635	713
635	714

FIG. 23

635	716
636	671
636	672
636	673
636	682
636	685
636	687
636	688
636	689
636	697
636	702
636	704
636	705
636	708
636	709
636	710
636	712
636	713
636	716
636	717
636	723
636	728
637	668
637	672
637	684

FIG. 23

637	688
637	697
637	702
637	704
637	705
637	706
637	709
637	712
637	713
637	716
638	685
638	698
638	702
638	713
639	670
639	682
639	684
639	686
639	698
639	702
639	703
639	704
639	707
639	708
639	709

FIG. 23

639	712
639	715
639	728
640	688
640	729
641	672
641	673
641	680
641	681
641	686
641	687
641	688
641	698
641	704
641	705
641	706
641	709
641	712
641	713
641	715
641	717
641	725
642	671
642	673
642	674

FIG. 23

642	678
642	679
642	683
642	684
642	687
642	688
642	699
642	700
642	703
642	704
642	705
642	708
642	710
642	711
642	712
642	713
642	714
642	716
642	717
642	719
642	720
642	722
642	723
643	675
643	677

FIG. 23

643	678
643	680
643	687
643	689
643	701
643	703
643	706
643	708
643	709
643	710
643	713
643	717
643	722
644	674
644	675
644	677
644	678
644	679
644	682
644	684
644	687
644	688
644	693
644	696
644	697

FIG. 23

644	698
644	701
644	702
644	703
644	704
644	706
644	707
644	708
644	710
644	711
644	712
644	713
644	714
644	715
644	716
644	717
644	722
644	725
645	674
645	675
645	676
645	677
645	678
645	679
645	680

FIG. 23

645	682
645	683
645	684
645	686
645	687
645	689
645	690
645	693
645	696
645	697
645	698
645	699
645	701
645	702
645	703
645	705
645	706
645	707
645	708
645	709
645	710
645	711
645	712
645	713
645	714

FIG. 23

645	715
645	716
645	717
645	719
645	725
645	728
645	735
645	739
646	675
646	677
646	678
646	679
646	680
646	683
646	684
646	686
646	687
646	688
646	690
646	692
646	698
646	701
646	702
646	703
646	704

FIG. 23

646	711
646	713
646	714
646	716
646	717
646	719
646	725
646	729
646	736
646	738
647	678
647	680
647	682
647	684
647	686
647	687
647	688
647	689
647	693
647	697
647	699
647	700
647	701
647	702
647	704

FIG. 23

647	705
647	706
647	708
647	710
647	712
647	713
647	714
647	717
647	719
647	720
647	722
647	729
647	730
647	731
647	732
647	734
648	677
648	678
648	679
648	680
648	682
648	683
648	684
648	686
648	687

FIG. 23

648	688
648	689
648	690
648	692
648	693
648	694
648	697
648	698
648	699
648	700
648	701
648	702
648	703
648	704
648	705
648	706
648	707
648	708
648	709
648	710
648	711
648	712
648	713
648	714
648	715

FIG. 23

648	716
648	717
648	718
648	719
648	722
648	723
648	729
648	730
648	732
648	736
648	737
648	738
649	679
649	680
649	685
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FIG. 23

649	705
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649	708
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649	724
649	727
650	680
650	684
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650	700
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FIG. 23

650	708
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650	716
650	717
650	731
651	681
651	682
651	684
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651	687
651	697
651	700
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FIG. 23

651	710
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FIG. 23

652	715
652	716
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653	684
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653	687
653	688
653	692
653	693
653	697
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653	711
653	713
653	714
653	716

FIG. 23

653	720
653	723
653	725
653	729
653	736
653	739
654	683
654	684
654	686
654	687
654	693
654	697
654	699
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654	702
654	703
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654	708
654	710
654	711
654	713
654	714
654	715
654	719
654	725

FIG. 23

654	729
655	684
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655	703
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655	711
655	712
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655	714
655	715
655	719
655	722

FIG. 23

655	723
655	732
655	738
656	687
656	698
656	703
656	730
657	686
657	687
657	700
657	705
657	706
657	711
657	713
657	714
657	715
657	717
657	721
658	711
658	713
659	688
659	689
659	697
659	699
659	700

FIG. 23

659	703
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659	719
659	722
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660	689
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660	710
660	712
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660	717
660	724

FIG. 23

660	729
660	731
660	732
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661	700
661	702
661	703
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FIG. 23

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662	729
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FIG. 23

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FIG. 23

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666	718
666	721
666	723
666	729
666	731
667	701
667	717
668	698
668	701
668	704
668	705
668	706
668	714
668	717
668	721
668	726
669	706

FIG. 23

669	710
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670	716
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670	736
671	705
671	712
671	713
671	715
671	720
672	704
672	705
672	706
672	710
672	711
672	717
672	720
672	725
672	736
673	704
673	705

FIG. 23

673	706
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674	711
674	712
674	713
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674	717
674	718
674	719
674	721

FIG. 23

674	724
674	729
674	731
674	735
675	704
675	705
675	708
675	717
675	724
675	725
675	727
675	738
676	709
676	712
676	714
676	715
676	722
676	723
676	729
677	706
677	716
677	720
678	712
678	713
678	717

FIG. 23

678	721
678	724
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FIG. 23

681	729
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684	725
684	730
685	717
685	722
685	730
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685	738
686	715
686	717
686	718
686	722
687	717
687	719
687	720

FIG. 23

687	722
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689	719
689	720
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689	728
689	736
690	723
691	730
693	725
693	729
699	729
699	731
699	739

FIG. 24A

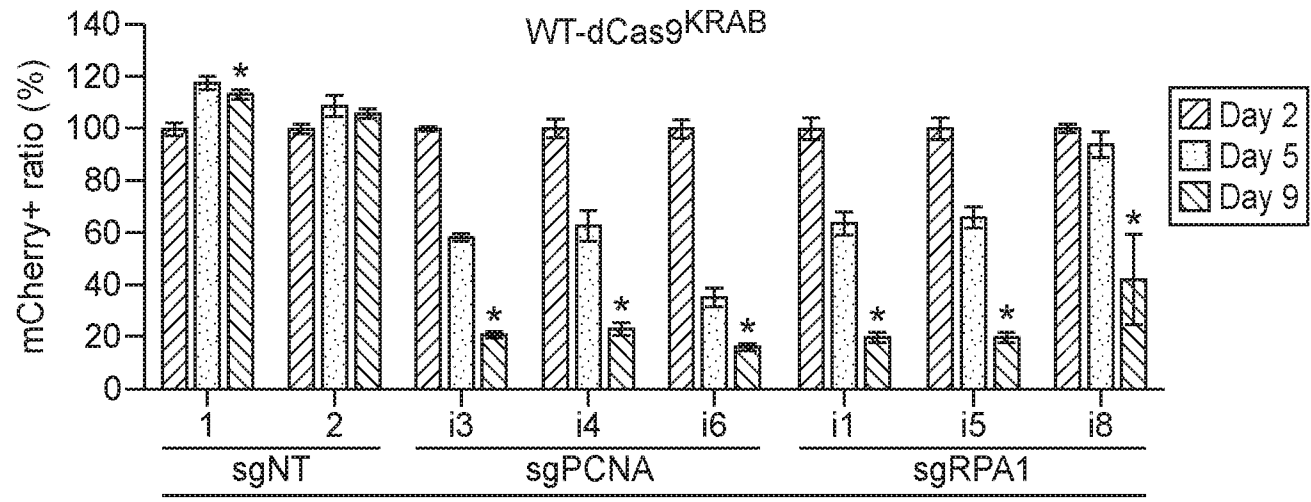


FIG. 24B

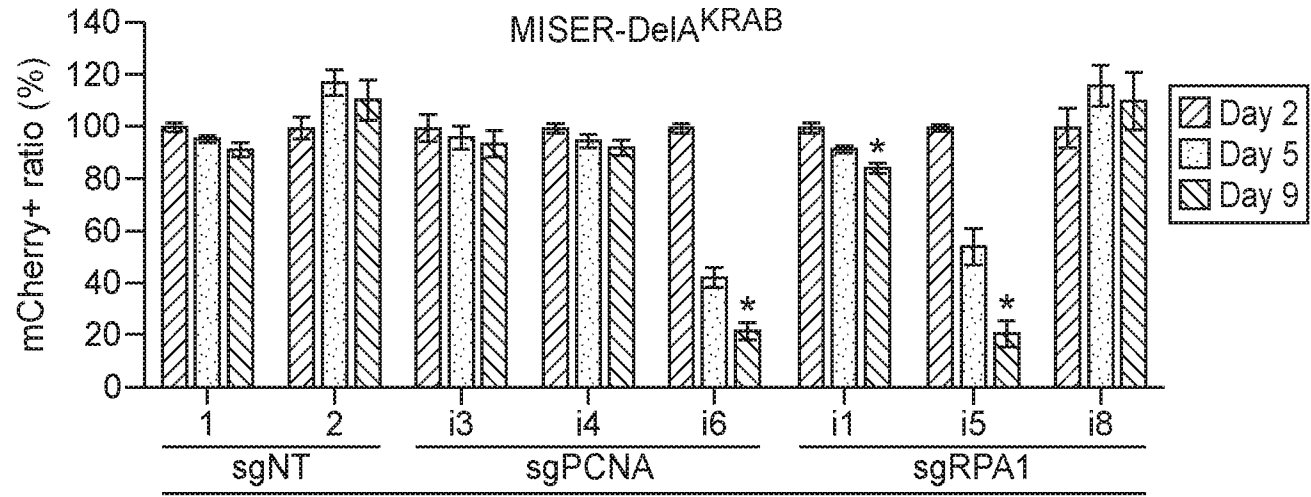


FIG. 24C

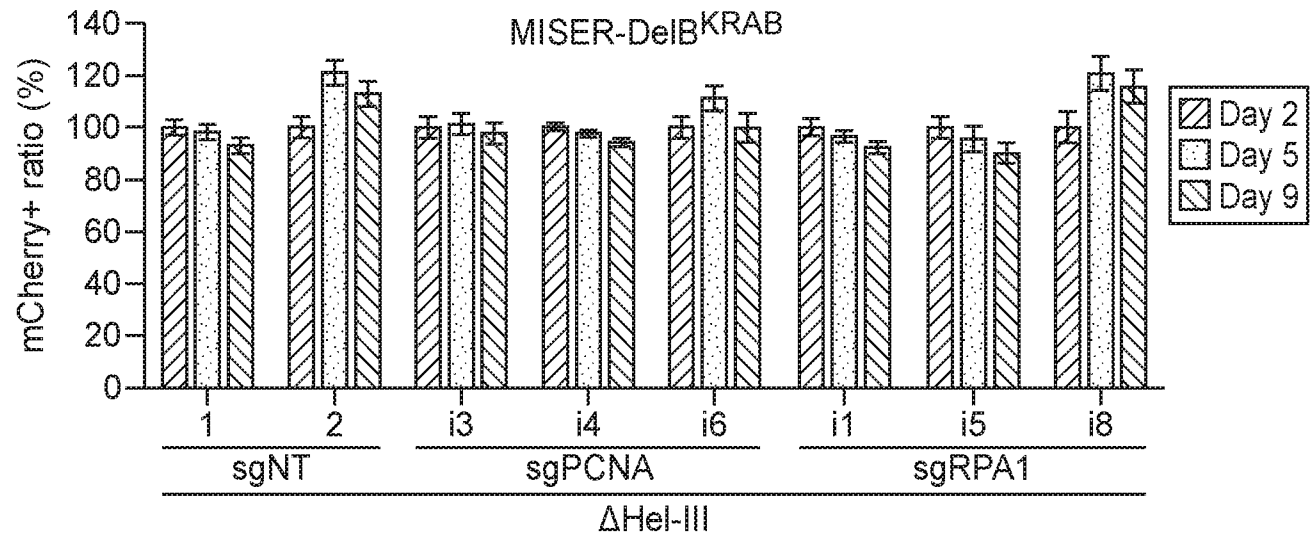


FIG. 24D

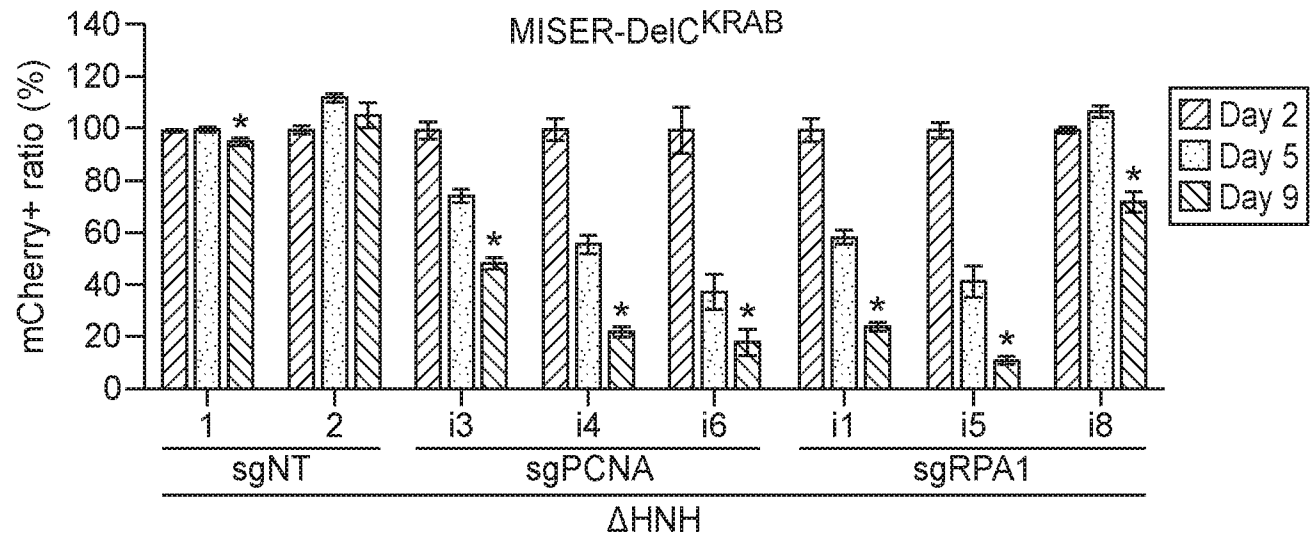
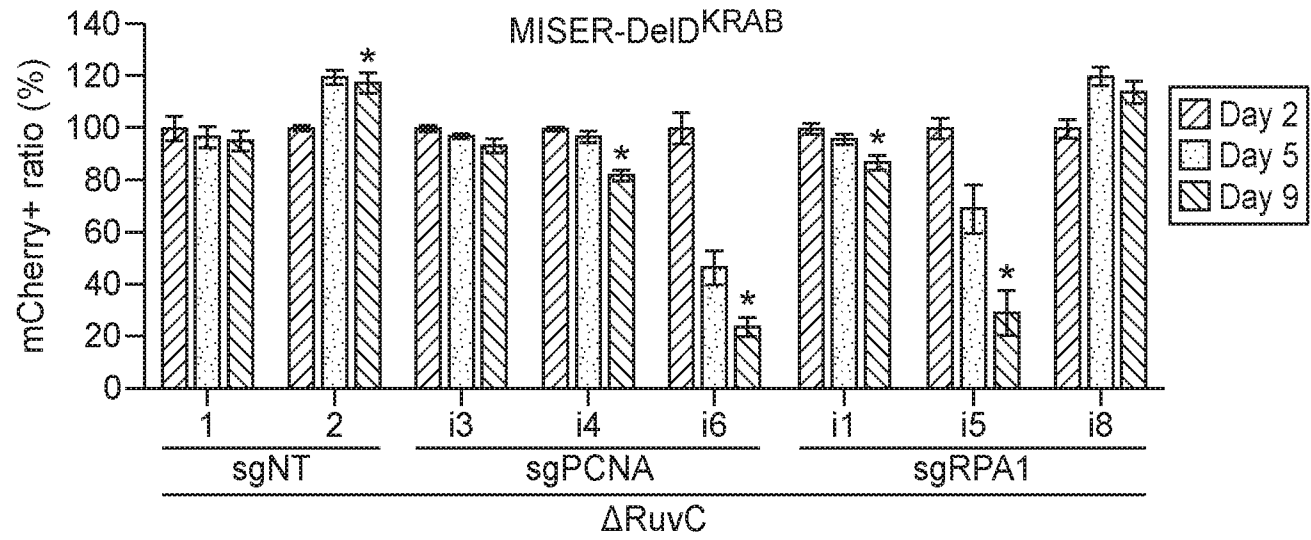


FIG. 24E



25

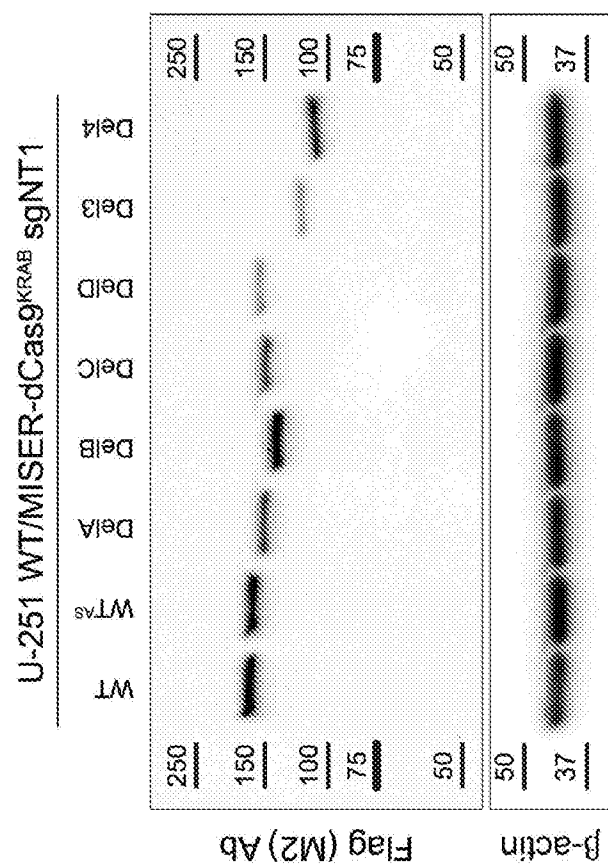


FIG. 26

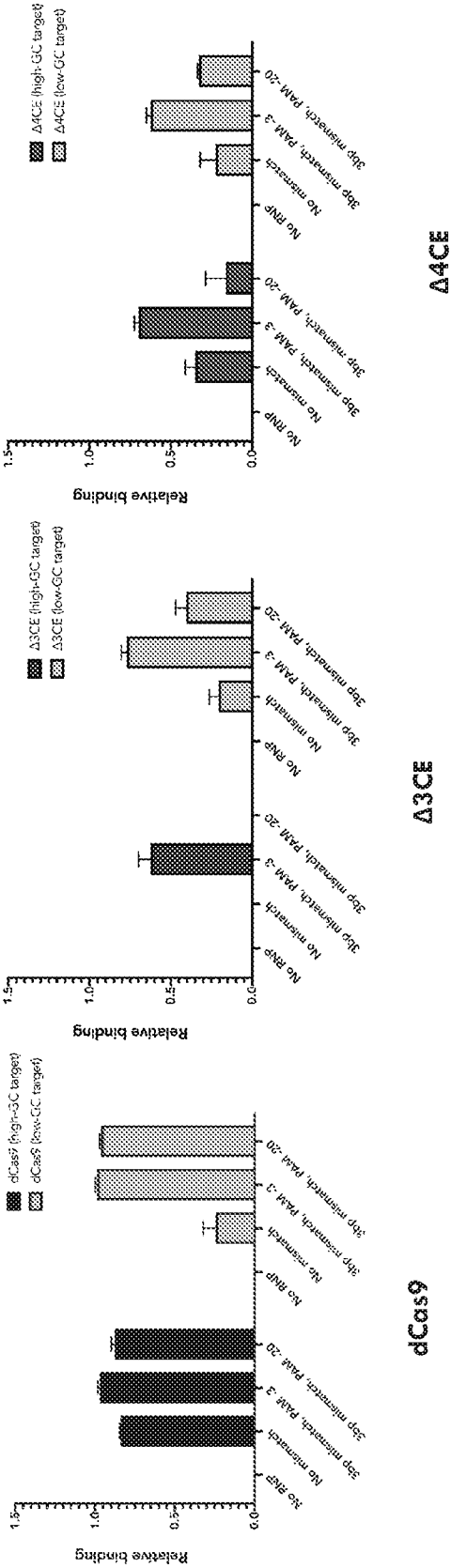
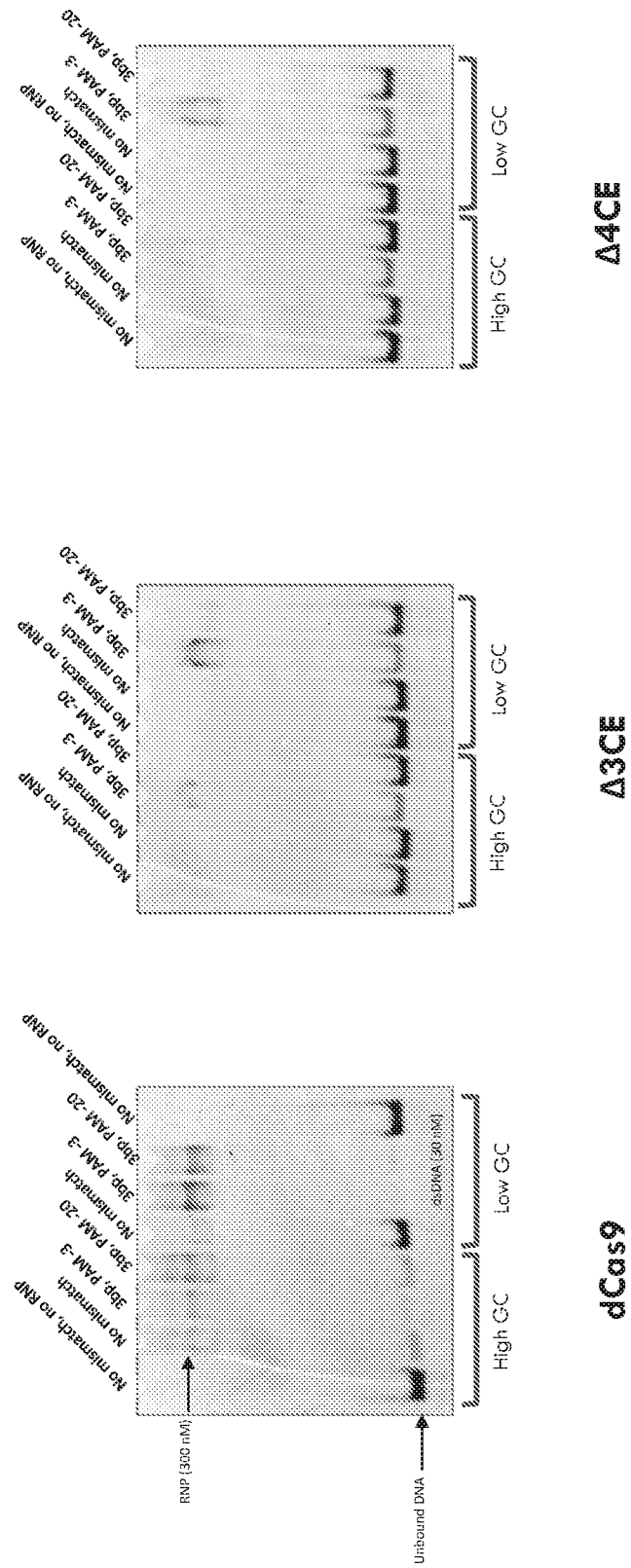


Fig. 27



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/39041

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 9/22; C12N 15/00 (2019.01)

CPC - C12N 9/22; C12N 15/00; C12N 15/102; C12N 2310/20

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)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2016/0340660 A1 (THE BROAD INSTITUTE, INC.) 24 November 2016 (24.11.2016). Especially para [0012], [0014], [0106], [0108], [0133], [0155], [0160], [0173], SEQ ID NO: 173	1, 2, 16, (24-28, 32, 33, 43-46, 56, 57)/(1,2,16) ----- 47/(1,2,16)
Y	US 5,981,273 A (CURIEL et al.) 9 November 1999 (09.11.1999). Especially col 5 ln 18-21	47/(1,2,16)

☐

Further documents are listed in the continuation of Box C.

☐

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

23 September 2019

Date of mailing of the international search report

25 NOV 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/39041

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item I.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. ☒ forming part of the international application as filed:

☒ 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 13ter. 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 13ter. 1(a)).

☐ on paper or in the form of an image file (Rule 13ter. 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:

GenCore ver 6.4.1 SEQ ID NO: 5 was searched

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/39041

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.: 29-31, 39, 48-55, 58-77
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:
-----Go to Extra Sheet for continuation-----

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 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.:
Claims 1, 2, 16, (24-28, 32, 33, 43-47, 56, 57) (in part), limited to deletion of amino acids 179-296 of SEQ ID NO: 5 and fusion partner regulates gene expression

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/39041

Continuation of Box III: Observations where Unity of Invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-28, 32-38, 40-47, 56-57, drawn to an RNA-guided effector polypeptide comprising one or more deletions comprising at least 4 contiguous amino acids.

The RNA-guided effector polypeptide will be searched to the extent that the deletion is the first named deletion, (i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324 where the amino acid numbering is based on SEQ ID NO: 5 (claim 1), specifically a deletion of amino acids 179-296 based on SEQ ID NO: 5 (claim 16) and the RNA-guided effector polypeptide is a fusion protein, where the heterologous fusion partner provides for control of gene expression (claim 33). It is believed that claims 1, 2, 16, (24-28, 32, 33, 43-47, 56, 57) (in part), read on this first named invention and thus these claims will be searched without fee to the extent that they a deletion of amino acids 166-294 based on the amino acid numbering of SEQ ID NO: 5 and the RNA-guided effector polypeptide is a fusion protein, where the heterologous fusion partner provides for control of gene expression. Additional amino acid deletions and fusion partners will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected amino acid deletions and fusion partners. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: a deletion of amino acids 503-708 (claim 17) based on the numbering of SEQ ID NO: 5 and the fusion partner is base editor cytidine deaminase (claims 40, 41) (claims 1, 3, 17, (24-28, 32, 40, 41, 43-47, 56, 57) (in part)).

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Among the inventions listed as Groups I+ are the specific RNA-guided effector polypeptide amino acid deletions and fusion partners recited therein. Each invention requires a specific defined polypeptide amino acid deletion and fusion partner not required by any other inventions.

Common Technical Features:

Group I+ inventions share the common technical feature of claim 1.

However, said common technical feature do not represent a contribution over the prior art, and is disclosed by US 2016/0340660 A1 to The Broad Institute, Inc. (hereinafter "Broad")

As to claim 1, Broad discloses an RNA-guided effector polypeptide having a length of from about 610 amino acids to about 1310 amino acids (para [0160]; 1,236 AA total size with deletion delta.175-307), wherein the RNA-guided effector polypeptide comprises one or more of:

i) a deletion of from 5 contiguous amino acids to 158 contiguous amino acids of amino acids 166-324 (para [0160]; "The REC lobe is one of the least conserved regions across the three families of Cas9 within the Type II CRISPR system (IIA, IIB and IIC) and many Cas9s contain significantly shorter REC lobes (FIGS. 18, 19). Applicants hypothesized that truncations in the REC lobe could be tolerated. As expected, and consistent with the observation that the REC2 domain does not contact the bound sgRNA:DNA hybrid duplex, a Cas9 mutant lacking the REC2 domain (delta.175-307) showed 50% of the wild-type Cas9 activity (FIG. 9B), indicating that the REC2 domain is not critical for DNA cleavage. The lower cleavage efficiency may be attributed in part to the reduced levels of Cas9 (delta.175-307) expression relative to that of the wild-type protein (FIG. 9C)"; delta.175-307 =132 amino acids deleted).

wherein the amino acid numbering is based on SEQ ID NO: 5 (SEQ ID NO: 174; 1368 AA 100% sequence identity; para [0155]. In this example, Applicants report the crystal structure of *S. pyogenes* Cas9").

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Group I+ inventions lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued): Claims 29-31, 39, 48-55, 58-77 are multiple dependent claims and are not drafted according to the second and third sentences of PCT Rule 6.4(a).