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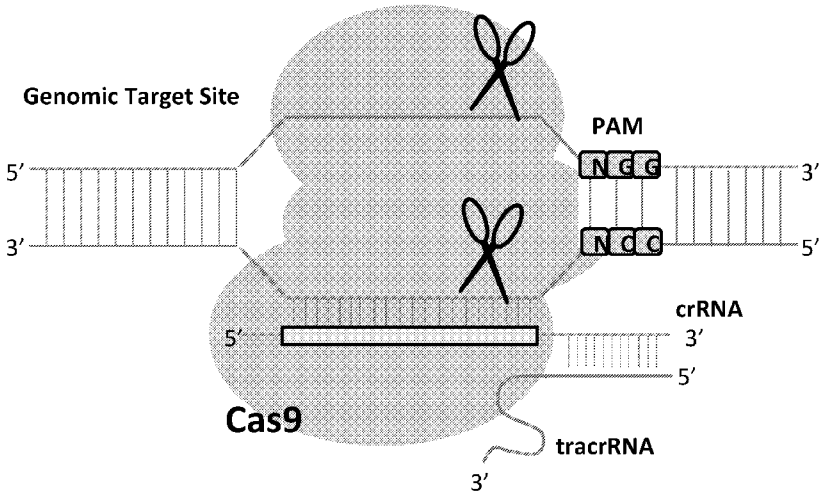
(54) Title: ALLELE SELECTIVE GENE EDITING AND USES THEREOF

FIG. 2



(57) Abstract: This invention encompasses compounds, structures, compositions and methods for therapeutic guide molecules that direct CRISPR gene editing. A guide molecule for directing gene editing can be allele selective, or disease allele selective, and can exhibit reduced off target activity. A guide molecule can be composed of monomers, including UNA monomers, nucleic acid monomers, and modified nucleotides, wherein the compound is targeted to a genomic DNA. The guide molecules of this invention can be used as active ingredients for editing or disrupting a gene in vitro, ex vivo, or in vivo.

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ALLELE SELECTIVE GENE EDITING AND USES THEREOF

TECHNICAL FIELD OF THE INVENTION

[0001] This invention relates to the fields of biopharmaceuticals and therapeutics for editing genes, and regulating gene expression. More particularly, this invention relates to methods and compositions for editing or altering a polynucleotide, including genomic polynucleotides, and ultimately, for in vivo gene editing, and modulating, disrupting, activating or repressing gene expression.

SEQUENCE LISTING

[0002] This application includes a Sequence Listing submitted electronically as an ASCII file named ARC5237WO_SL.txt.

BACKGROUND OF THE INVENTION

[0003] Gene editing that is specific for a predetermined site can be done with the target-guided nuclease Cas9 and polynucleotide repair methods. Using the target-guided Cas9 endonuclease, both strands of a double stranded DNA can be cut near a target site to create a double-strand break.

[0004] The target specificity of Cas9 is determined by a guide molecule, which complexes Cas9 to the polynucleotide target. Polynucleotide target sequences, typically 17-20 bases in length, must be flanked by a 3' protospacer-adjacent motif (PAM). The structure of PAM is determined by the species of bacteria from which the Cas9 was derived. Fortuitously, suitable target sequences containing a PAM can be found in most genes of interest in most species. In one variation, the guide molecule can be made as a single RNA strand that has a sequence complementary to the target, which is attached to a bacterially-derived crispr-tracr RNA sequence that complexes Cas9.

[0005] In some modalities, after forming a double-strand break in dsDNA at a specific site, the break can be repaired to achieve editing of the DNA. A double-strand break can be repaired by non-homologous end joining (NHEJ) to generate random insertions and deletions. A double-strand break can also be repaired by homology-

directed repair (HDR) using an exogenous DNA template to generate controlled insertions, deletions, and substitutions.

[0006] A major drawback of gene editing with Cas9 is that the guide molecule may have limited effectiveness for a target polynucleotide. The specificity and activity of a guide molecule can be unpredictable. Guide molecules for Cas9 editing can vary widely in effectiveness, and some guides that otherwise follow the structural scheme can be ineffective.

[0007] A further drawback of gene editing with Cas9 is that the guide molecule may lack selectivity for a target allele. Variations in the genome can contribute to disease conditions. Some alleles related to disease phenotypes have been identified in medical genetics. The inability to target particular alleles is a significant drawback of current methods for of gene editing.

[0008] Other drawbacks of gene editing with CRISPR-Cas systems include the occurrence of off-target mutations.

[0009] What is needed are stable and effective guide molecules for gene editing, as well as compositions and methods for use in treating disease.

[0010] There is an urgent need for new molecules for guiding gene editing with Cas9, and for allele selectivity and reduced off target activity.

BRIEF SUMMARY

[0011] This invention provides guide molecules that can be highly effective for CRISPR gene editing. The compositions and methods of this invention can be used for gene editing in vivo, ex vivo, and in vitro.

[0012] This invention further contemplates methods for gene editing with a Cas enzyme guided by novel allele-selective guide molecules. In some embodiments, guide molecules of this invention can used to perform gene editing with CRISPR-Cas systems with reduced occurrence of off-target mutations.

[0013] Guide molecules of this invention can provide efficient gene editing using Cas9. The Guide molecules of this invention can be active for gene editing to select

between allelic variations based on one or more nucleotide polymorphisms. Further advantages of guide molecules of this disclosure include reduced off-target effects.

[0014] In some embodiments, the guide molecules of this invention can exhibit an extraordinary and surprising level of allele selectivity for targeting genomic DNA and generating double strand breaks through CRISPR/Cas gene editing. In certain embodiments, guide molecules of this invention can provide reduced off-target activity and greater efficiency of gene editing.

[0015] This invention also contemplates methods for gene editing with Cas guided by guide molecules, along with gene repair by any mechanism, including NHEJ and HDR repair mechanisms.

[0016] The guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas.

[0017] In some embodiments, the guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas9 and provide a high frequency of targeted mutagenesis via NHEJ.

[0018] In further embodiments, the guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas9 and provide exact DNA integration using HDR for any genomic target.

[0019] In some aspects, the guide molecules of this invention can enhance Cas9 binding and DNA cleavage in vivo.

[0020] This invention further provides novel molecules to be used as therapeutic agents for various diseases and conditions. The molecules of this invention can be used as active pharmaceutical ingredients in compositions for ameliorating, preventing or treating various diseases and conditions.

[0021] In some aspects, this invention provides guide molecules having structures that may include various combinations of linker groups, chain-forming monomers, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides, as well as certain natural nucleotides. These guide molecules can exhibit allele selectivity for

targeting genomic DNA. This disclosure provides guide molecules that can used to perform CRISPR-Cas gene editing with reduced off-target mutations.

[0022] Embodiments of this invention include the following:

[0023] A guide compound targeted to a genomic DNA, comprising a target guide chain of 14-24 contiguous monomers attached to a crRNA, wherein the guide compound directs CRISPR gene editing of the genomic DNA.

[0024] The guide compound above, wherein the monomers comprise UNA monomers and nucleic acid monomers, and wherein the guide compound comprises a sequence of bases targeted to direct CRISPR gene editing of the genomic DNA.

[0025] The guide compound above, wherein the sequence of bases of the target guide chain has up to three mismatches from the genomic DNA.

[0026] The guide compound above, wherein the guide compound contains one to five UNA monomers.

[0027] The guide compound above, wherein the nucleic acid monomers are selected from natural nucleotides, non-natural nucleotides, modified nucleotides, chemically-modified nucleotides, and combinations thereof.

[0028] The guide compound above, wherein one or more of the nucleic acid monomers is a 2'-O-methyl ribonucleotide, a 2'-O-methyl purine nucleotide, a 2'-deoxy-2'-fluoro ribonucleotide, a 2'-deoxy-2'-fluoro pyrimidine nucleotide, a 2'-deoxy ribonucleotide, a 2'-deoxy purine nucleotide, a universal base nucleotide, a 5-C-methyl-nucleotide, an inverted deoxyabasic monomer residue, a 3'-end stabilized nucleotide, a 3'-glyceryl nucleotide, a 3'-inverted abasic nucleotide, a 3'-inverted thymidine, a locked nucleic acid nucleotide (LNA), a 2'-O,4'-C-methylene-(D-ribofuranosyl) nucleotide, a 2'-methoxyethoxy (MOE) nucleotide, a 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotide, a 2'-O-methyl nucleotide, a 2′,4′-Constrained 2′-O-Methoxyethyl (cMOE), a 2′-O-Ethyl (cEt), a 2'-amino nucleotide, a 2'-O-amino nucleotide, a 2'-C-allyl nucleotides, a 2'-O-allyl nucleotide, a N^6-methyladenosine nucleotide, a nucleotide with modified base 5-(3-amino)propyluridine, a nucleotide with modified base 5-(2-mercapto)ethyluridine, a nucleotide with modified base 5-bromouridine, a nucleotide with modified base 8-

bromoguanosine, a nucleotide with modified base 7-deazaadenosine, a 2'-O-aminopropyl substituted nucleotide, or a nucleotide with a 2'-OH group replaced with a 2'-R, a 2'-OR, a 2'-halogen, a 2'-SR, or a 2'-amino, where R can be H, alkyl, alkenyl, or alkynyl.

[0029] The guide compound above, wherein one or more of the last three monomers at each end of the guide compound is connected by a phosphorothioate, a chiral phosphorothioate, or a phosphorodithioate linkage.

[0030] The guide compound above, wherein the guide compound directs double strand breaks in a gene selected from TTR, BIRC5, CDK16, STAT3, CFTR, F9, KRAS, and CAR.

[0031] The guide compound above, wherein the genomic DNA contains a target disease-related single nucleotide polymorphism.

[0032] The guide compound above, wherein the guide compound directs double strand breaks in a disease-related allele.

[0033] The guide compound above, wherein the guide compound directs double strand breaks in a disease-related allele selected from V30M TTR, G284R ColA1, L132P Keratin12, R135T Keratin12, G85R SOD1, G272V Tau, P301L Tau, V337M Tau, R406W Tau, Q39STOP beta-Globin, T8993G/C mtDNA, G719S EGFR, and G12C Kras.

[0034] The guide compound above, comprising 30-300 contiguous monomers.

[0035] The guide compound above, wherein the CRISPR gene editing uses Cas9.

[0036] The guide compound above, wherein the guide compound directs gene editing with reduced off target activity.

[0037] The guide compound above, wherein the guide compound directs more double strand breaks in a disease-related allele than in the same allele as a wild type.

[0038] A guide compound above annealed with a tracrRNA.

[0039] The guide compound above, wherein the tracrRNA is derived from *S. pneumonia*, *S. pyogenes*, *N. menigiditis,* or *S. thermophiles*.

[0040] A guide compound above annealed with a tracrRNA and complexed with a CRISPR-associated gene editing protein.

[0041] The guide compound above, wherein the CRISPR-associated gene editing protein is Cas9.

[0042] A guide compound targeted to a genomic DNA, wherein the guide compound is a chain of monomers and directs CRISPR gene editing of the genomic DNA, the guide compound comprising a target guide chain, a CRISPR crRNA, and a CRISPR tracrRNA as a single strand, wherein the target guide chain is 14-24 contiguous monomers in length, wherein the monomers comprise UNA monomers and nucleic acid monomers, and wherein the guide compound comprises a sequence of bases targeted to direct CRISPR gene editing of the genomic DNA.

[0043] The guide compound above, wherein the guide compound directs gene editing in a CRISPR/Cas9 complex.

[0044] A pharmaceutical composition comprising one or more guide compounds above and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier may comprise a viral vector or a non-viral vector. The pharmaceutically acceptable carrier may comprise liposomes.

[0045] Embodiments of this invention include methods for editing a genomic DNA in a cell, wherein the cell comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising contacting the cell with a composition above.

[0046] The method above, wherein the editing is disrupting the DNA or repressing transcription of the DNA. The method above, wherein the editing is achieved with reduced off target activity. The method above, wherein the CRISPR gene editing enzyme is co-transfected with a composition above.

[0047] This invention includes methods for editing a genomic DNA in a subject in vivo, wherein the subject comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising administering to the subject a composition above. The editing can be disrupting the DNA or repressing transcription of the DNA. The editing can be achieved with reduced off target activity. The CRISPR gene editing enzyme may be co-transfected with a composition above.

[0048] This invention further contemplates methods for preventing, treating or ameliorating a disease associated with a target genomic DNA in a subject in need, wherein the subject comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising administering to the subject a composition above.

[0049] In some embodiments, this invention describes the use of a composition above for preventing, ameliorating or treating a disease or condition in a subject in need, the use in medical therapy, the use in the treatment of the human or animal body, or the use of a composition above for preparing or manufacturing a medicament for preventing, ameliorating or treating a disease or condition in a subject in need.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] FIG. 1: FIG. 1 illustrates a CRISPR-Cas gene editing complex with a "single guide" structure.

[0051] FIG. 2: FIG. 2 illustrates a CRISPR-Cas gene editing complex.

[0052] FIG. 3: Allele selective gene editing of a transthyretin (TTR) genomic site with a U-Guide molecule for CRISPR/Cas9. FIG. 3 shows that U-Guide molecules UNA1 and UNA2 directed the cleavage of a 357-bp genomic TTR DNA at a predetermined position shown by the appearance of 275-bp and 82-bp products. As shown in FIG. 3, the U-Guide molecules of this invention exhibited surprisingly high allele selective gene editing of human V30M TTR over wild type TTR. This indicates the capability for reduced off target activity. Further, under the same conditions a CRISPR/Cas9 cr/tracr comparative guide (gRNA) having the same nucleobase sequence and structure as the U-Guide molecule, but lacking a UNA monomer, exhibited some selectivity for human V30M TTR over wild type TTR.

[0053] FIG. 4: FIG. 4 shows that the U-Guide molecules UNA1 and UNA2 of this invention provided selective editing of V30M TTR over wild type TTR in a CRISPR/Cas9 system. The U-Guide molecules UNA1 and UNA2 produced high levels of double strand breaks in V30M TTR (patterned bar), but surprisingly few double strand breaks in wild type TTR (black bar). Thus, the U-Guide molecules UNA1 and UNA2 of this invention were extraordinarily active for allele selective gene editing of human TTR.

This indicates the capability for reduced off target activity. The Neg control contained no CRISPR/tracr guide.

[0054] FIG. 5: The U-Guide molecules of this invention can be used for allele selective gene editing of human TTR. The surprising level of allele selectivity for gene editing of human TTR is shown in FIG. 5. The U-Guide molecules UNA1 and UNA2 provided high selectivity ratios of 8.7 and 9.5, respectively. This indicates the capability for reduced off target activity. Further, under the same conditions, a CRISPR/Cas9 cr/tracr guide (gRNA) having the same nucleobase sequence and structure as the U-Guide molecules, but lacking any UNA monomer, exhibited selectivity ratio of 1.4. Thus, the U-Guide molecules UNA1 and UNA2 were extraordinarily active for gene editing human TTR with allele selectivity of V30M TTR over wild type TTR.

[0055] FIG. 6: Fig. 6 shows the indel spectrum for a comparative gRNA guide (non-UNA guide structure) for assessment of genome editing of V30M TTR by sequence trace decomposition (TIDE).

[0056] FIG. 7: Fig. 7 shows the indel spectrum for UNA-guide (UNA1) for assessment of genome editing of V30M TTR by sequence trace decomposition (TIDE).

[0057] FIG. 8: Fig. 8 shows the indel spectrum for a comparative gRNA guide (non-UNA guide structure) for assessment of genome editing of Wild Type TTR by sequence trace decomposition (TIDE).

[0058] FIG. 9: Fig. 9 shows the indel spectrum for UNA-guide (UNA1) for assessment of genome editing of Wild Type TTR by sequence trace decomposition (TIDE).

[0059] FIG. 10: Allele selective gene editing of a transthyretin (TTR) genomic site with a U-Guide molecule for CRISPR/Cas9. FIG. 10 shows that a U-Guide molecule UNA3 directed the cleavage of a 357-bp genomic TTR DNA at a predetermined position shown by the appearance of 271-bp and 86-bp products. As shown in FIG. 10, the U-Guide molecule of this invention exhibited allele selective gene editing of human V30M TTR over wild type TTR. This indicates the capability for reduced off target activity. Further, under the same conditions a CRISPR/Cas9 guide (gRNA) having the same

nucleobase sequence and structure as the U-Guide molecule, but lacking any UNA monomer, exhibited some selectivity.

[0060] FIG. 11: FIG. 11 shows that a U-Guide molecule UNA3 of this invention provided selective editing of V30M TTR over wild type TTR in a CRISPR/Cas9 system. The U-Guide molecule UNA3 produced high levels of double strand breaks in V30M TTR (patterned bar), but surprisingly few double strand breaks in wild type TTR (black bar). Thus, the U-Guide molecule UNA3 of this invention was extraordinarily active for allele selective gene editing of human TTR. This indicates the capability for reduced off target activity. The Neg control contained no CRISPR/tracr guide.

[0061] FIG. 12: The U-Guide molecules of this invention can be used for allele selective gene editing of human TTR. The surprising level of allele selectivity for gene editing of human TTR is shown in FIG. 12. The U-Guide molecule UNA3 provided high a selectivity ratio of 4.7. This indicates the capability for reduced off target activity. Further, under the same conditions, a CRISPR/Cas9 guide (gRNA) having the same nucleobase sequence and structure as the U-Guide molecule, but lacking any UNA monomer, exhibited a selectivity ratio of 1.3. Thus, the U-Guide molecule UNA3 was extraordinarily active for gene editing human TTR with allele selectivity of V30M TTR over wild type TTR.

[0062] FIG. 13: Fig. 13 shows a schematic representation of the structure of a chimeric antigen receptor (CAR). ScFv is a single chain fragment variable. V_H is a heavy-chain variable region. V_L is a light-chain variable region. TM is a transmembrane domain. SD is a signaling domain.

[0063] FIG. 14: Fig. 14 shows a schematic of a method for introducing a CAR gene into a constitutive CD2 gene of a T cell, in which the CAR is downstream from the CD2. A double strand break is made with a U-Guide molecule of this invention. The gene inserted by homologous recombination can be comprised of a section of CD2, along with P2A and the CAR section. P2A peptide is a self-cleaving peptide that can be used to generate the two separate gene products CD2 protein and CAR protein. The CAR protein receptor can carry the specificity of a mAb against cancer cells of a subject in an adoptive immunotherapy strategy to kill the subject's cancer cells.

[0064] FIG. 15: Fig. 15 shows a schematic of a method for introducing a CAR gene into a constitutive CD2 gene of a T cell, in which the CAR is upstream from the CD2.

DETAILED DESCRIPTION OF THE INVENTION

[0065] This invention provides a range of novel agents and compositions to be used for gene editing and therapeutic applications. Molecules of this invention can be used as guide components for compositions taking advantage of CRISPR gene editing modalities. The molecules and compositions of this invention can be used for ameliorating, preventing or treating various diseases associated with genes and their functionalities.

[0066] Guide molecules of this invention can provide efficient gene editing using Cas9, Cas9, and other gene editing enzymes.

[0067] The Guide molecules of this invention can be active for gene editing human genes. A Guide molecule can be attached to, or annealed with a tracrRNA to provide a Guide/tracr molecule for CRISPR/ Cas gene editing.

[0068] The Guide/tracr molecules of this invention can be delivered and transfected into cells in vitro, in vivo, or ex vivo for editing a genomic DNA.

[0069] The Guide molecules of this invention can be surprisingly active for gene editing human genes with allele selective results.

[0070] In some embodiments, the Guide molecules of this invention exhibit an extraordinary and surprising level of allele selectivity for gene editing and generating double strand breaks in genomic DNA. This indicates the capability for advantageously reduced off target activity.

[0071] In some aspects, the ability to create double strand breaks in genomic DNA includes the ability to alter, modulate, or reduce the expression of the DNA in a cell.

[0072] A cell may be a eukaryotic cell, a mammalian cell, or a human cell.

[0073] The Guide molecules of this invention can be used for allele selective gene editing of human genomic DNA. This disclosure provides guide molecules that can used to perform CRISPR-Cas gene editing with reduced off-target mutations.

[0074] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human variant allele over a corresponding wild type allele with reduced off target effect.

[0075] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity of at least 30% as measured by editing efficiency.

[0076] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity of at least 40% as measured by editing efficiency.

[0077] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity ratio of at least 2 as measured by editing efficiency.

[0078] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity ratio of at least 3 as measured by editing efficiency.

[0079] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity ratio of at least 5 as measured by editing efficiency.

[0080] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with a selectivity ratio of at least 8 as measured by editing efficiency.

[0081] By comparison, under the same conditions, a CRISPR/Cas9 guide having a selectivity ratio of 1 indicates lack of selectivity.

[0082] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with essentially no off target activity toward the wild type allele.

[0083] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with less than 1% off target activity toward the wild type allele.

[0084] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with less than 3% off target activity toward the wild type allele.

[0085] The properties of the guide compounds of this invention arise according to their molecular structure, and the structure of the molecule in its entirety, as a whole, can provide significant benefits based on those properties. Embodiments of this invention can provide guide molecules having one or more properties that advantageously provide enhanced effectiveness in gene editing with Cas9, as well as compositions or formulations for therapeutic agents for various diseases and conditions, which can provide clinical agents.

[0086] A wide range of novel guide molecules are provided herein, each of which can incorporate specialized linker groups. The linker groups can be attached in a chain in the guide molecule. Each linker group can also be attached to a nucleobase.

[0087] In some aspects, a linker group can be a monomer. Monomers can be attached to form a chain molecule. In a chain molecule of this invention, a linker group monomer can be attached at any point in the chain.

[0088] In certain aspects, linker group monomers can be attached in a chain molecule of this invention so that the linker group monomers reside near the ends of the chain. The ends of the chain molecule can be formed by linker group monomers.

[0089] As used herein, a chain molecule can also be referred to as an oligomer.

[0090] In further aspects, the linker groups of a chain molecule can each be attached to a nucleobase. The presence of nucleobases in the chain molecule can provide a sequence of nucleobases.

[0091] In certain embodiments, this invention provides oligomer guide molecules having chain structures that incorporate novel combinations of the linker group

monomers, along with certain natural nucleotides, or non-natural nucleotides, or modified nucleotides, or chemically-modified nucleotides.

[0092] The oligomer guide molecules of this invention can display a sequence of nucleobases that is targeted to at least a portion of a gene. In some embodiments, an oligomer can be targeted to at least a portion of a gene that is conserved, or highly conserved, among a number of variants.

[0093] In some aspects, this invention provides active oligomer guide molecules that correspond to, or are complementary to at least a fragment of a nucleic acid molecule, and that provide editing of at least such a fragment present in a cell.

[0094] In some embodiments, the cell can be a eukaryotic cell, a mammalian cell, or a human cell.

[0095] This invention provides structures, methods and compositions for oligomeric guide agents that incorporate the linker group monomers. The oligomeric guide molecules of this invention can be used as active agents in formulations for gene editing therapeutics.

[0096] This invention provides a range of guide molecules that are useful for providing therapeutic effects because of their activity in editing a gene. The guide molecules of this invention are structured to provide gene editing activity in vitro, ex vivo, and in vivo.

[0097] The guide molecules of this invention can be used in any CRISPR/Cas system.

[0098] In certain embodiments, an active guide molecule can be structured as an oligomer composed of monomers. The oligomeric structures of this invention may contain one or more linker group monomers, along with certain nucleotides.

[0099] In some aspects, this invention provides a CRISPR/Cas system having a Cas9 protein and one or more guide molecules that target a gene in a eukaryotic cell.

[00100] A guide molecule of this invention may have a guide sequence fused to a crispr-tracr sequence.

[00101] In further aspects, the CRISPR/Cas system may be used to cleave one or both strands of the DNA of the gene target.

[00102] The CRISPR gene editing enzyme, for example Cas9 protein, can be derived from *S. pneumonia*, *S. pyogenes* (for example, UniProtKB accession number Q99ZW2; CAS9_STRP1), *N. menigiditis,* and *S. thermophilus*, among other species.

[00103] The CRISPR gene editing enzyme may be derived from a genus including Corynebacter, Sutterella, Legionella, Treponemna, Filifactor, Eubacterium, Streptococcus, Lactobacillus, Mycoplasma, Bacteroides, Flaviivola, Flavobacterium, Sphaerochaeta, Azospirillumn, Gluconacetobacter, Neisseria, Roseburia, Parvibaculum, Staphylococcus, Nitratifractor, Mycoplasma, and Campylobacter.

[00104] Embodiments of this invention can include methods for altering, modulating or reducing expression of a gene product. In some embodiments, a eukaryotic cell may contain and be expressing a DNA molecule having a target sequence, where the DNA encodes the gene product. The cell can be transfected with an engineered, non-naturally occurring CRISPR-associated (Cas) system, including an inducible or constitutive guide molecule of this invention that hybridizes with the target sequence. The CRISPR-associated (Cas) system may further include an inducible or constitutive Type-II Cas9 protein. The CRISPR-associated (Cas) system may further include one or more nuclear localization signals. The guide molecule can locate the target sequence and direct the Cas protein to cleave the DNA, and expression of a gene product can be altered. The Cas protein and the guide molecule do not naturally occur together.

[00105] Vectors for providing expression of one or more sequences in mammalian cells are known in the art.

[00106] Some examples of a Cas protein include Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, and Cas9.

[00107] A CRISPR-associated gene editing protein can include a Cas protein.

[00108] A CRISPR gene editing system can include polynucleotides, transcripts and moieties involved in the expression of, or directing the activity of genes encoding a

CRISPR-associated (Cas) protein, a tracrRNA, and a guide chain. A CRISPR system can be derived from a particular organism having an endogenous CRISPR system, such as Streptococcus pyogenes. A CRISPR gene editing system can promote the formation of a CRISPR complex at the site of a target DNA sequence.

[00109] A Cas9 protein can be modified or mutated, or can be a homolog or ortholog for improved expression in a eukaryotic cell. A Cas9 protein can be human codon optimized. In some embodiments, paired guide molecules can be used to target different strands of a dsDNA with paired Cas9 nickases. Cleavage of both DNA strands by a pair of Cas9 nickases can be used to create a site-specific double strand break, which may decrease off-target effects without loss of efficiency of editing.

[00110] A guide molecule of this invention may contain a guide chain, which can also be referred to as a target guide chain. The guide chain can be composed of a chain of monomers, and each of the monomers can have an attached nucleobase. The guide chain can have a base sequence, which has sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence. The guide chain can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00111] A guide molecule of this invention may contain a guide chain having a base sequence with sufficient complementarity to a target polynucleotide sequence to hybridize with the target sequence. The guide molecule may further contain a CRISPR portion or crRNA attached to the guide chain, where the crRNA can bind to a tracrRNA and direct sequence-specific binding of a CRISPR complex to the target sequence. Thus, the guide molecule can be a guide chain attached to a crRNA to form the guide molecule.

[00112] In some embodiments, this invention includes "single guide" embodiments in which a guide chain having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence is attached to a crRNA sequence, which is further attached to a tracrRNA sequence, to form a "single guide molecule," where the single guide molecule can direct sequence-specific binding of a CRISPR complex to the target sequence. An example of a "single guide" embodiment is shown in Fig. 1.

[00113] A guide molecule of this invention, a crRNA, a guide chain, or a tracrRNA may contain one or more non-natural nucleotides, or modified nucleotides, or chemically-modified nucleotides.

[00114] In some embodiments, a guide molecule can be from 20 to 120 bases in length, or more. In certain embodiments, a guide molecule can be from 20 to 60 bases in length, or 20 to 50 bases, or 30 to 50 bases, or 39 to 46 bases.

[00115] In certain embodiments, a polynucleotide target sequence can be 5-100 bases in length, or 5-50 bases, or 5-30 bases, or 5-25 bases, or 5-24 bases, or 5-23 bases, or 5-22 bases, or 5-21 bases, or 5-20 bases, or 5-19 bases, or 5-18 bases.

[00116] In certain embodiments, a polynucleotide target sequence can be or 18-30 bases in length, or 18-24 bases, or 18-22 bases.

[00117] In additional embodiments, a polynucleotide target sequence can be 16 bases in length, or 17 bases, or 18 bases, or 19 bases, or 20 bases, or 21 bases, or 22 bases, or 23 bases, or 24 bases, or 25 bases, or 26 bases, or 27 bases, or 28 bases, or 29 bases, or 30 bases, or 31 bases, or 32 bases, or 33 bases, or 34 bases, or 35 bases.

[00118] In additional embodiments, a single guide molecule can be from 40 to 200 bases in length, or more.

[00119] The property of a guide sequence to direct sequence-specific binding of a CRISPR complex to a target sequence may be determined by any assay known in the art.

[00120] This invention further contemplates methods for delivering one or more vectors, or one or more transcripts thereof to a cell, as well as cells and organisms produced.

[00121] In some embodiments, the components of a CRISPR/Cas complex, including a guide molecule, can be delivered to a cell, in vitro, ex vivo, or in vivo. Viral and non-viral transfer methods as are known in the art can be used to introduce nucleic acids in mammalian cells. Nucleic acids can be delivered with a pharmaceutically acceptable vehicle, or for example, encapsulated in a liposome.

[00122] The target sequence can be any polynucleotide sequence, endogenous or exogenous to the eukaryotic cell. The target polynucleotide can be a coding or non-

coding sequence. The target sequence can be associated with a PAM sequence, as are known in the art.

[00123] The target sequence can be any disease-associated polynucleotide or gene, as have been established in the art.

[00124] This invention further contemplates methods and compositions for repairing breaks in a polynucleotide or gene.

[00125] In some embodiments, a break in a polynucleotide or gene can be repaired by non-homologous end joining (NHEJ) to generate random insertions and deletions. The method may result in one or more changes in the structure of a protein expressed from a repaired target gene.

[00126] In further embodiments, a break in a polynucleotide or gene can be repaired by homology-directed repair (HDR) using an exogenous polynucleotide template to generate controlled insertions, deletions, and substitutions. The method may result in one or more changes in the structure of a protein expressed from a repaired target gene.

[00127] The repair of a break in a polynucleotide or gene can be done with a sense or antisense, single stranded oligonucleotide as a repair template, as is known in the art.

[00128] Allele selective embodiments and reduced off target

[00129] This invention further contemplates Guide molecules that are allele selective for gene editing and generating double strand breaks in genomic DNA.

[00130] In some aspects, the Guide molecules of this invention can be used for gene editing with reduced off target activity.

[00131] In further aspects, the Guide molecules of this invention can be used for gene editing of a human gene variant allele over a corresponding wild type allele, with essentially no off target activity toward the wild type allele.

[00132] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with less than 1% off target activity toward the wild type allele.

[00133] In certain embodiments, the Guide molecules of this invention can be used for allele selective gene editing of a human gene variant allele over a corresponding wild type allele, with less than 3% off target activity toward the wild type allele.

[00134] An allele selective guide molecule of this invention may contain a guide chain. The guide chain can be composed of a chain of monomers, and each of the monomers can have an attached nucleobase. The guide chain can have a base sequence, which has sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence. The guide chain can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00135] A guide molecule of this invention having reduced off target effects may contain a guide chain. The guide chain can be composed of a chain of monomers, and each of the monomers can have an attached nucleobase. The guide chain can have a base sequence, which has sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence. The guide chain can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00136] An allele selective guide molecule of this invention may contain a guide chain having a base sequence with sufficient complementarity to a target polynucleotide sequence to hybridize with the target sequence. The guide molecule may further contain a CRISPR portion or crRNA attached to the guide chain, where the crRNA can bind to a tracrRNA and direct sequence-specific binding of a CRISPR complex to the target sequence. Thus, the guide molecule can be a guide chain attached to a crRNA to form the guide molecule.

[00137] A guide molecule of this invention exhibiting reduced off target effects may contain a guide chain having a base sequence with sufficient complementarity to a target polynucleotide sequence to hybridize with the target sequence. The guide molecule may further contain a CRISPR portion or crRNA attached to the guide chain, where the crRNA can bind to a tracrRNA and direct sequence-specific binding of a CRISPR complex to the target sequence. Thus, the guide molecule can be a guide chain attached to a crRNA to form the guide molecule.

[00138] In some embodiments, this invention includes allele selective "single guide" embodiments in which a guide chain having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence is attached to a crRNA sequence, which is further attached to a tracrRNA sequence, to form a "single guide molecule," where the single guide molecule can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00139] Examples of target polynucleotide sequences for guide molecules of this invention are shown in Table 1. The target polynucleotide sequences in Table 1 reflect single nucleotide polymorphisms in certain human genes, which are disease-related.

Table 1: Guide target sequences for single nucleotide polymorphisms in human genes

Gene	Mutation	Strand	20-mer target (5'-3')	PAM	Cas9
ColA1	G284R	(+)	aagggagaagcc**a**gagatcc	NGG	S. pyr
	G284R	(+)	gcc**a**gagatcctggaagacc	NGG	S. pyr
	G284R	(+)	cc**a**gagatcctggaagaccc	NGG	S. pyr
	G284R	(+)	c**a**gagatcctggaagacccg	NGG	S. pyr
	G284R	(-)	c**t**ggcttctcccttctctcc	NGG	S. pyr
Keratin 12	L132P	(+)	aaactatgcaaaatc**c**taat	NNNNG ATT	N. menigiditis
	R135T	(+)	tgata**c**attagcttcctacc	NGG	S. pyr
SOD1	G85R	(+)	g**c**gcaatgtgactgctgacaaaga (24-mer)	NGG	S. pyr
Tau	G272V	(+)	gcaccagccgggag**t**cggga	NGG or NGGNG	S. thermophilus
	G272V	(+)	tgaagcaccagccgggag**t**c	NGG	S. pyr
	G272V	(+)	ctgaagcaccagccgggag**t**	NGG	S. pyr
	G272V	(-)	cg**a**ctcccggctggtgcttc	NGG	S. pyr
	G272V	(-)	gcaccttcccg**a**ctcccggc	NGG or NGGNG	S. pyr or S. thermophilus

Gene	Mutation	Strand	20-mer target (5'-3')	PAM	Cas9
	G272V	(-)	atctgcaccttcccg**a**ctcc	NGG	S. pyr
	P301L	(+)	gataatatcaaacacgtcc**t**	NGG or NGGNG	S. pyr or S. thermophilus
	P301L	(+)	aatatcaaacacgtcc**t**ggg	NGG	S. pyr
	V337M	(-)	acttcca**t**ctggccacctcc	NGG	S. pyr
	V337M	(-)	tctcagattttacttcca**t**c	NGG	S. pyr
	V337M	(-)	ca**t**ctggccacctcctggtttatg (24-mer)	NNGRR (R = A/G)	SaCas
	R406W	(-)	gagacattgctgagatgcc**a**	NGG or NGGNG	S. pyr or S. thermophilus
beta-Globin	Q39STOP	(+)	tggtctacccttggacc**t**ag	NGG	S. pyr
	Q39STOP	(+)	**t**cagaggttctttgagtcctt	NGG	S. pyr
	Q39STOP	(+)	cccttggacc**t**agaggttct	NNGRR	SaCas
	Q39STOP	(-)	tcaaagaacctct**t**ggtcca	NGG	S. pyr
	Q39STOP	(-)	caaagaacctct**t**ggtccaa	NGG	S. pyr
	Q39STOP	(-)	ctcaaagaacctct**t**ggtcc	NNGRR	SaCas
mtDNA	T8993G/C	(+)	agcggttaggcgtacggcc(**c/g**)	NGG	S. pyr
	T8993G/C	(+)	aggcgtacggcc**(c/g)**gggctat	NGG	S. pyr
	T8993G/C	(+)	cgtacggcc**(c/g)**gggctattgg	NNGRR	SaCas
	T8993G/C	(+)	cggcc(**c/g**)gggctattggttga	NNGRR	SaCas
EGFR	G719S	(+)	aagatcaaagtgctg**a**gctc	NGG or NGGNG	S. pyr or S. thermophilus
	G719S	(+)	gtgctg**a**gctccggtgcgtt	NGG	S. pyr
	G719S	(+)	g**a**gctccggtgcgttcggca	NGG or NGGNG	S. pyr or S. thermophilus

Gene	Mutation	Strand	20-mer target (5'-3')	PAM	Cas9
	G719S	(-)	agct**t**cagcactttgatcttt	NNGRR	SaCas
Kras	G12C		cttgtggtagttggagct**t**g	NGG	

[00140] In Table 1, the position of the single nucleotide allelic mutation is underlined.

Table 2: Accession numbers for gene targets

Disease	Gene	NCBI Acc #	Mutation
Ullrich Congenital Muscular Dystrophy (UCMD)	COL6A1	NM_001848.2	G284R (GGA to AGA)
Meesmann epithelial corneal dystrophy (MECD)	KRT12	NM_000223.3	L132P (CTT to CCT) and/or R135T (AGA to ACA)
Amyotrophic lateral sclerosis (ALS)	SOD1	NM_000454.4	G85R (GGC to CGC)
Frontotemporal dementia with parkinsonism linked to chromosom 17 (FTDP-17)	Tau	NM_001123066.3	G272V (GGC to GTC), P301L (CCG to CTG), V337M (GTG to ATG), and/or R406W (CGG to TGG)
b-Thalassaemia	HBB	NM_000518.4	Q39STOP (CAG to TAG)
Neurogenic weakness, ataxia and retinitis pigmentosa (NARP)	MT-ATP6	NC_012920.1	T8993G/C
Gefitinib-resistant cancer	EGFR	NM_005228.3	G719S

[00141] This invention contemplates Guide molecules that are allele selective for gene editing and generating double strand breaks in disease-related single nucleotide polymorphisms in human genes.

[00142] This invention further contemplates Guide molecules for gene editing and generating double strand breaks in disease-related single nucleotide polymorphisms in human genes with reduced off target activity.

[00143] An allele selective guide molecule of this invention may contain a guide chain. The guide chain can be composed of a chain of monomers, and each of the monomers can have an attached nucleobase. The guide chain can have a base sequence, which has sufficient complementarity with a target polynucleotide sequence containing a single nucleotide polymorphism to hybridize with the target sequence. The guide chain can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00144] An allele selective guide molecule of this invention may contain a guide chain having a base sequence with sufficient complementarity to a target polynucleotide sequence containing a single nucleotide polymorphism to hybridize with the target sequence. The guide molecule may further contain a CRISPR portion or crRNA attached to the guide chain, where the crRNA can bind to a tracrRNA and direct sequence-specific binding of a CRISPR complex to the target sequence. Thus, the guide molecule can be a guide chain attached to a crRNA to form the guide molecule.

[00145] In some embodiments, this invention includes allele selective "single guide" embodiments in which a guide chain having sufficient complementarity with a target polynucleotide containing a single nucleotide polymorphism sequence to hybridize with the target sequence is attached to a crRNA sequence, which is further attached to a tracrRNA sequence, to form a "single guide molecule," where the single guide molecule can direct sequence-specific binding of a CRISPR complex to the target sequence.

[00146] TTR embodiments

[00147] Amyloidosis related to transthyretin (ATTR) involves the depositing of amyloid fibril proteins in various organs and tissues, including the peripheral, autonomic, and central nervous systems. Transthyretin (TTR) is a secreted thyroid hormone-binding protein that binds and transports retinol binding protein, and serum thyroxine in plasma and cerebrospinal fluid.

[00148] The pathology of ATTR may include many TTR mutations. Symptoms of ATTR often include neuropathy and/or cardiomyopathy. Peripheral neuropathy can

begin in the lower extremities, with sensory and motor neuropathy, and can progress to the upper extremities. Autonomic neuropathy can be manifest by gastrointestinal symptoms and orthostatic hypotension.

[00149] Patients with TTR gene Val-30-Met, the most common mutation, have normal echocardiograms. However, they may have conduction system irregularities and need a pacemaker. The ATTR V30M variant can cause lower extremity weakness, pain, and impaired sensation, as well as autonomic dysfunction. Vitreous and opaque amyloid deposits can be characteristic of ATTR.

[00150] The U-Guide molecules of this invention can be active for gene editing human TTR. A U-Guide molecule can be attached to, or annealed with a tracrRNA to provide a U-Guide/tracr molecule for CRISPR/ Cas9 gene editing.

[00151] The U-Guide/tracr molecules of this invention can be delivered and transfected into cells in vitro, in vivo, or ex vivo for editing a genomic DNA.

[00152] The U-Guide molecules of this invention can be surprisingly active for gene editing human TTR with allele selective results.

[00153] In some embodiments, a U-Guide molecule of this invention can be active for gene editing human TTR with reduced off target activity.

[00154] In some embodiments, the U-Guide molecules of this invention exhibit an extraordinary and surprising level of allele selectivity for generating double strand breaks in V30M TTR over wild type TTR.

[00155] The U-Guide molecules of this invention can be used for allele selective gene editing of human TTR.

[00156] In further embodiments, the U-Guide molecules of this invention can be used for allele selective gene editing of human V30M TTR over wild type TTR with a selectivity ratio of at least 3.

[00157] In further embodiments, the U-Guide molecules of this invention can be used for allele selective gene editing of human V30M TTR over wild type TTR with a selectivity ratio of at least 5.

[00158] In additional embodiments, the U-Guide molecules of this invention can be used for allele selective gene editing of human V30M TTR over wild type TTR with a selectivity ratio of at least 8.

[00159] By direct comparison, under the same conditions, a CRISPR/Cas9 guide having the same nucleobase sequence and structure as the U-Guide molecule, but lacking any UNA monomer, may have a selectivity ratio of about 1, or less than 2.

[00160] In further aspects, the U-Guide molecules of this invention can be used for gene editing of human V30M TTR over wild type TTR, with essentially no off target activity toward the wild type allele.

[00161] In certain embodiments, the U-Guide molecules of this invention can be used for gene editing of human V30M TTR over wild type TTR, with less than 1% off target activity toward the wild type allele.

[00162] In certain embodiments, the U-Guide molecules of this invention can be used for gene editing of human V30M TTR over wild type TTR, with less than 3% off target activity toward the wild type allele.

[00163] U-guide molecules

[00164] This invention further provides U-guide molecules that can be highly effective for gene editing with Cas9. The compositions and methods of this invention can be used for gene editing with Cas9 in vivo, ex vivo, and in vitro.

[00165] This invention contemplates methods for gene editing with Cas9 guided by novel U-guide molecules.

[00166] U-Guide molecules of this invention can provide efficient gene editing using Cas9.

[00167] The U-Guide molecules of this invention can be active for gene editing a TTR gene. The U-Guide molecules of this invention can be surprisingly active for gene editing human TTR with allele selective results, and can exhibit reduced off target effects.

[00168] In some embodiments, the U-Guide molecules of this invention exhibit an extraordinary and surprising level of allele selectivity for generating double strand breaks in V30M TTR over wild type TTR, indicating reduced off target effects.

[00169] This invention further contemplates methods for gene editing with Cas9 guided by novel U-guide molecules, along with gene repair by NHEJ and HDR repair mechanisms.

[00170] The U-guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas9.

[00171] In some embodiments, the U-guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas9 and provide a high frequency of targeted mutagenesis via NHEJ.

[00172] In further embodiments, the U-guide molecules of this invention can advantageously increase the efficiency of gene engineering directed by Cas9 and provide exact DNA integration using HDR for any genomic target.

[00173] In some aspects, the U-guide molecules of this invention can enhance Cas9 binding and DNA cleavage in vivo.

[00174] This invention provides novel molecules to be used as therapeutic agents for various diseases and conditions. The molecules of this invention can be used as active pharmaceutical ingredients in compositions for ameliorating, preventing or treating various diseases and conditions.

[00175] In some embodiments, molecules of this invention can be used for ameliorating and/or treating amyloidosis and related amyloid-related diseases, or Alzheimer's Disease.

[00176] Embodiments of this invention can provide guide molecules that advantageously provide effective gene editing with Cas9, as well as compositions or formulations for therapeutic agents, which can provide clinical agents.

[00177] The properties of the guide molecules of this invention arise according to their structure, and the molecular structure in its entirety, as a whole, can provide significant benefits and properties.

[00178] In some embodiments, a wide range of novel U-guide molecules are provided, which can incorporate one or more linker groups. The linker groups can be attached in a chain in the guide molecule. Each linker group can also be attached to a nucleobase.

[00179] In some aspects, a linker group can be a monomer. Monomers can be attached to form a chain molecule. In a chain molecule of this invention, a linker group monomer can be attached at any point in the chain.

[00180] In certain aspects, linker group monomers can be attached in a chain molecule of this invention so that the linker group monomers reside near the ends of the chain. The ends of the chain molecule can be formed by linker group monomers.

[00181] In further aspects, the linker groups of a chain molecule can each be attached to a nucleobase. The presence of nucleobases in the chain molecule can provide a sequence of nucleobases.

[00182] In certain embodiments, this invention provides oligomer molecules having chain structures that incorporate novel combinations of the linker group monomers, along with certain natural nucleotides, or non-natural nucleotides, or modified nucleotides, or chemically-modified nucleotides.

[00183] The oligomer molecules of this invention can display a sequence of nucleobases that is targeted to at least a portion of a polynucleotide or genome.

[00184] This invention provides structures, methods and compositions for oligomeric agents that incorporate the linker group monomers. The oligomeric molecules of this invention can be used as active agents in formulations for gene editing therapeutics.

[00185] Modalities of action

[00186] Embodiments of this invention can provide an active guide molecule, which can be used for altering or editing a gene in a cell, thereby modulating gene functionality, gene expression or gene expression products.

[00187] This invention can provide robust and efficient methods for gene editing with a wide range of therapeutic applications.

[00188] In general, the CRISPR/Cas system can utilize a guide molecule to recognize a specific DNA target. The Cas enzyme may be recruited to a specific DNA target by the action of the guide molecule. The CRISPR/Cas system can be used for efficient and effective gene editing using guide molecules of this invention.

[00189] In some aspects, this invention provides methods for altering or modulating expression of one or more gene products.

[00190] Methods of this invention may utilize a vector for introducing into a eukaryotic cell the components of the Type II CRISPR/Cas9 Guided-Endonuclease gene editing system. The vector can have a regulatory sequence operably linked to a guide molecule that can hybridize with a target sequence in a gene, and an additional regulatory sequence operably linked to a Type II Cas9 endonuclease. The guide molecule can recruit the Cas9 protein to cleave the gene target. In certain embodiments, the vector can include a nuclear localization signal.

[00191] Some information concerning vectors is given in, for example, David V. Goeddel (Editor), Methods in Enzymology, Volume 185: Gene Expression Technology, Academic Press, 1990.

[00192] In some embodiments, a guide molecule may have a guide sequence attached to a crispr-tracr sequence. The guide sequence can be targeted to hybridize a gene target, and the crispr-tracr sequence can bind to Cas9.

[00193] Without wishing to be bound by any particular theory, a Type II prokaryotic CRISPR and CRISPR-associated protein (Cas) system can be used for gene editing. In the prokaryote, the system operates as an immune defense system. The CRISPR gene can consist of certain repeat sequences separated by spacer sequences that belong to targeted foreign genes. A primary transcript from CRISPR can be processed into CRISPR RNAs (crRNAs). The crRNA can consist of a conserved repeat sequence, and a variable spacer sequence or guide that is complementary to the target gene sequence. Trans activating crisper RNA (tracrRNA) can be a short RNA sequence that is complementary to the CRISPR repeat and serves to process crRNA. The complex formed by crRNA, tracrRNA and Cas9 binds to a target sequence by base pairing and causes sequence-specific, double strand DNA cleavage.

[00194] In further embodiments, a guide molecule of this invention can encompass structures that incorporate sequences related to crRNA and tracrRNA.

[00195] A CRISPR/Cas complex may include a guide sequence hybridized to a target sequence and complexed with a Cas protein. The CRISPR/Cas complex can provide cleavage of one or both strands of the target sequence, or within a few base pairs of the target sequence, or near the target sequence.

[00196] The components of the CRISPR/Cas complex including the Cas protein, the guide sequence, and the tracr sequence may each be operably linked to separate regulatory sequences on separate vectors.

[00197] The components of the CRISPR/Cas complex may be expressed from the same or different regulatory sequences, and may be combined in a single vector.

[00198] A vector may be used to provide one or more guide sequences.

[00199] As used herein, the term "Cas" refers to any Cas protein known in the art that is operable for gene editing using a guide molecule.

[00200] In some embodiments, one or more guide sequences can be used simultaneously for gene editing.

[00201] In some embodiments, this invention provides methods and compositions for knocking out genes, for amplifying genes, for repairing mutations associated with genomic instability, and for correcting known defects in a genome.

[00202] In some embodiments, the expression of one or more gene products of the target gene can be decreased.

[00203] In certain embodiments, the expression of one or more gene products of the target gene can be increased.

[00204] In some modalities, a CRISPR/Cas system can utilize a guide molecule of this invention for CRISPR genomic interference.

[00205] In certain aspects, a CRISPR/Cas system can utilize a guide molecule of this invention to repress gene expression. A catalytically inactive Cas9 can be used to suppress gene expression by interfering with transcription of the gene. A guide molecule

of this invention can target the inactive Cas9 to a genomic sequence, acting as a repressor. The guide molecule may be co-expressed.

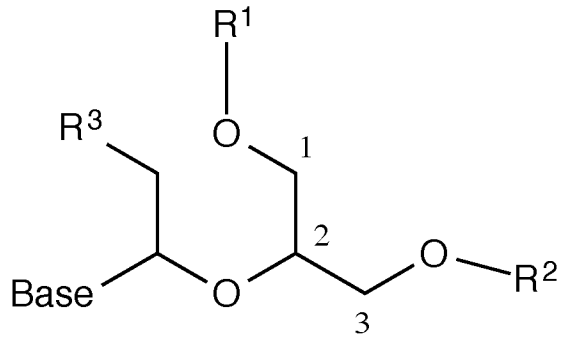
[00206] In certain embodiments, attachment of an effector domain having regulatory function to an inactive Cas9 can provide stable and efficient transcriptional repression. Attachment of a transcriptional repressor domain or regulatory domain having regulatory function to an inactive Cas9 can suppress expression of a targeted endogenous gene.

[00207] In some embodiments, a guide molecule of this invention can be relatively short, up to 14 or 16 nt in length, to allow an active Cas9 to bind specific target sequences without cleaving the DNA, therefore acting as a repressor.

[00208] In further aspects, a CRISPR/Cas system can utilize a guide molecule of this invention to activate gene expression. A transcriptional activator can be attached to an inactive Cas9. The transcriptional activator can increase gene expression, while the inactive Cas9 is targeted with a guide molecule of this invention.

[00209] UNA monomers

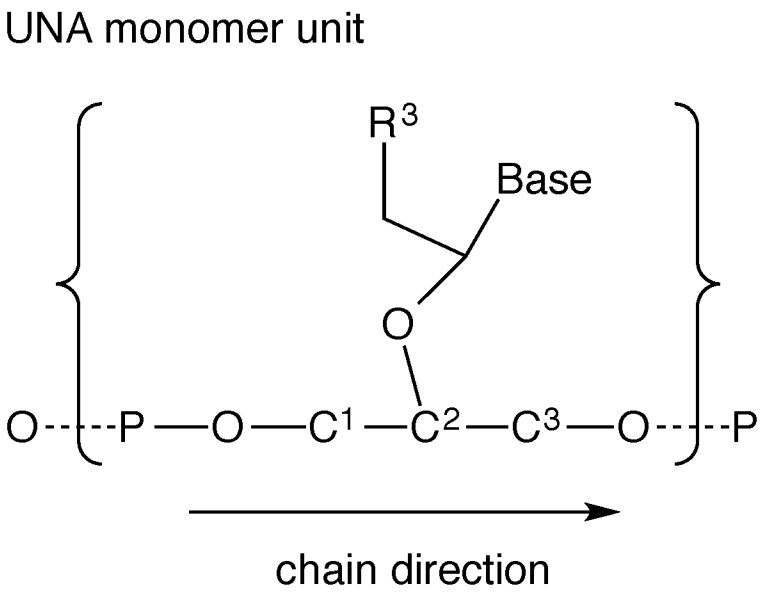
In some embodiments, linker group monomers can be unlocked nucleomonomers (UNA monomers), which are small organic molecules based on a propane-1,2,3-tri-yl-trisoxy structure as shown below:



UNA MONOMER

where R^1 and R^2 are H, and R^1 and R^2 can be phosphodiester linkages, Base can be a nucleobase, and R^3 is a functional group described below.

[00210] In another view, the UNA monomer main atoms can be drawn in IUPAC notation as follows:

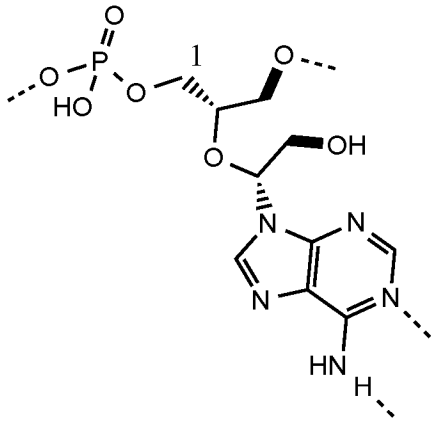


where the direction of progress of the oligomer chain is from the 1-end to the 3-end of the propane residue.

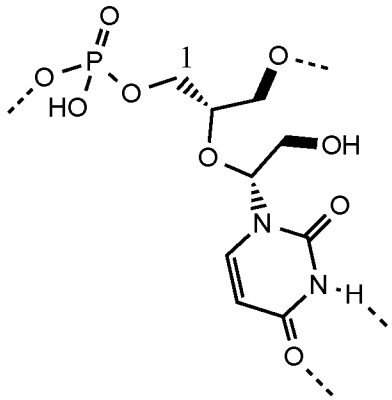
[00211] Examples of a nucleobase include uracil, thymine, cytosine, 5-methylcytosine, adenine, guanine, inosine, and natural and non-natural nucleobase analogues.

[00212] In general, because the UNA monomers are not nucleotides, they can exhibit at least four forms in an oligomer. First, a UNA monomer can be an internal monomer in an oligomer, where the UNA monomer is flanked by other monomers on both sides. In this form, the UNA monomer can participate in base pairing when the oligomer is a duplex, for example, and there are other monomers with nucleobases in the duplex.

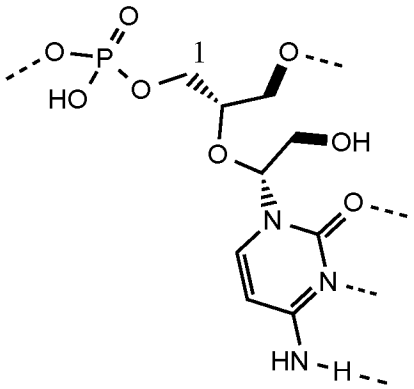
[00213] Examples of UNA monomer as internal monomers flanked at both the propane-1-yl position and the propane-3-yl position, where R^3 is -OH, are shown below.



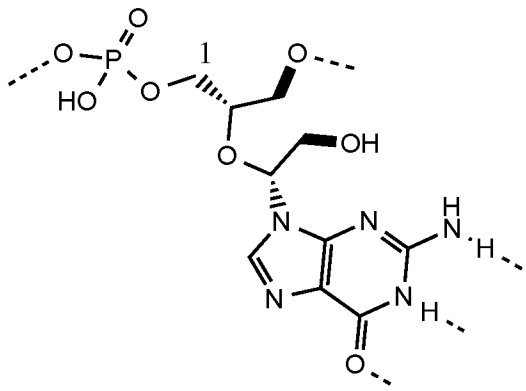
UNA-A



UNA-U



UNA-C



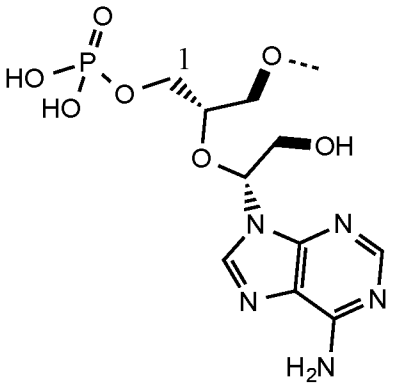
UNA-G

[00214] Second, a UNA monomer can be a monomer in an overhang of an oligomer duplex, where the UNA monomer is flanked by other monomers on both sides. In this form, the UNA monomer does not participate in base pairing. Because the UNA monomers are flexible organic structures, unlike nucleotides, the overhang containing a UNA monomer will be a flexible terminator for the oligomer.

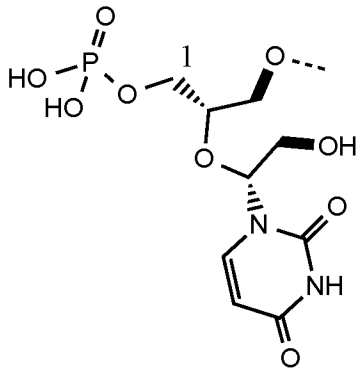
[00215] A UNA monomer can be a terminal monomer in an overhang of an oligomer, where the UNA monomer is attached to only one monomer at either the propane-1-yl position or the propane-3-yl position. In this form, the UNA monomer does not participate in base pairing. Because the UNA monomers are flexible organic

structures, unlike nucleotides, the overhang containing a UNA monomer can be a flexible terminator for the oligomer.

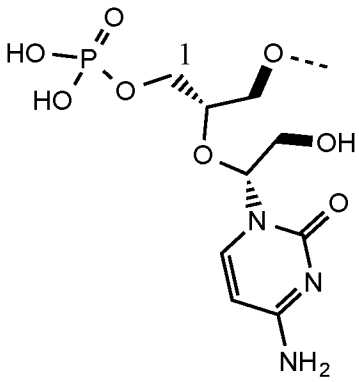
[00216] Examples of a UNA monomer as a terminal monomer attached at the propane-3-yl position are shown below.



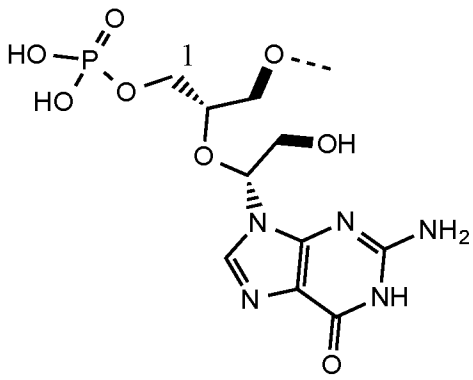
terminal UNA-A



terminal UNA-U

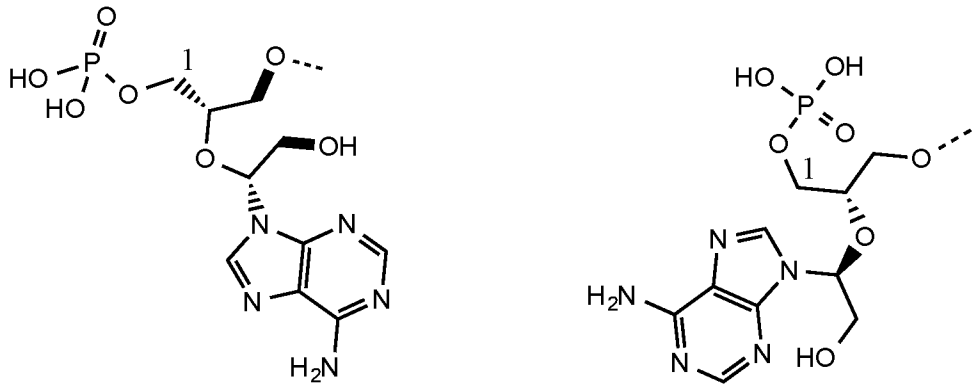


terminal UNA-C



terminal UNA-G

[00217] Because a UNA monomer can be a flexible molecule, a UNA monomer as a terminal monomer can assume widely differing conformations. An example of an energy minimized UNA monomer conformation as a terminal monomer attached at the propane-3-yl position is shown below.



UNA-A terminal forms: the dashed bond shows the propane-3-yl attachment

Thus, UNA oligomers having a terminal UNA monomer are significantly different in structure from conventional nucleic acid agents. In contrast, the conformability of a terminal UNA monomer can provide UNA oligomers with different properties.

[00218] Among other things, the structure of the UNA monomer allows it to be attached to naturally-occurring nucleotides. A UNA oligomer can be a chain composed of UNA monomers, as well as various nucleotides that may be based on naturally-occurring nucleosides.

[00219] In some embodiments, the functional group R^3 of a UNA monomer can be —OR^4, —SR^4, —NR^4_2, —NH(C=O)R^4, morpholino, morpholin-1-yl, piperazin-1-yl, or 4-alkanoyl-piperazin-1-yl, where R^4 is the same or different for each occurrence, and can be H, alkyl, a cholesterol, a lipid molecule, a polyamine, an amino acid, or a polypeptide.

[00220] The UNA monomers are organic molecules. UNA monomers are not nucleic acid monomers or nucleotides, nor are they naturally-occurring nucleosides or modified naturally-occurring nucleosides.

[00221] A UNA oligomer of this invention is a synthetic chain molecule. A UNA oligomer of this invention is not a nucleic acid, nor an oligonucleotide.

[00222] In some embodiments, as shown above, a UNA monomer can be UNA-A (designated Ã), UNA-U (designated Ũ), UNA-C (designated Č), and UNA-G (designated Ğ).

[00223] Designations that may be used herein include mA, mG, mC, and mU, which refer to the 2'-O-Methyl modified ribonucleotides.

[00224] Designations that may be used herein include lower case c and u, which refer to the 2'-O-methyl modified ribonucleotides.

[00225] Designations that may be used herein include dT, which refers to a 2'-deoxy T nucleotide.

[00226] Additional monomers for guide compounds

[00227] As used herein, in the context of oligomer sequences, the symbol X represents a UNA monomer.

[00228] As used herein, in the context of oligomer sequences, the symbol N represents any natural nucleotide monomer, or a modified nucleotide monomer.

[00229] As used herein, in the context of oligomer sequences, the symbol Q represents a non-natural, modified, or chemically-modified nucleotide monomer.

[00230] When a Q monomer appears in one strand of a duplex, and is unpaired with the other strand, the monomer can have any base attached. When a Q monomer appears in one strand of a duplex, and is paired with a monomer in the other strand, the Q monomer can have any base attached that would be complementary to the monomer in the corresponding paired position in the other strand.

[00231] Examples of nucleic acid monomers include non-natural, modified, and chemically-modified nucleotides, including any such nucleotides known in the art.

[00232] Examples of non-natural, modified, and chemically-modified nucleotide monomers include any such nucleotides known in the art, for example, 2'-O-methyl ribonucleotides, 2'-O-methyl purine nucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 2'-deoxy-2'-fluoro pyrimidine nucleotides, 2'-deoxy ribonucleotides, 2'-deoxy purine

nucleotides, universal base nucleotides, 5-C-methyl-nucleotides, and inverted deoxyabasic monomer residues.

[00233] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 3'-end stabilized nucleotides, 3'-glyceryl nucleotides, 3'-inverted abasic nucleotides, and 3'-inverted thymidine.

[00234] Examples of non-natural, modified, and chemically-modified nucleotide monomers include locked nucleic acid nucleotides (LNA), 2'-O,4'-C-methylene-(D-ribofuranosyl) nucleotides, 2'-methoxyethoxy (MOE) nucleotides, 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotides, and 2'-O-methyl nucleotides.

[00235] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2′,4′-Constrained 2′-O-Methoxyethyl (cMOE) and 2′-O-Ethyl (cEt) Modified DNAs.

[00236] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-amino nucleotides, 2'-O-amino nucleotides, 2'-C-allyl nucleotides, and 2'-O-allyl nucleotides.

[00237] Examples of non-natural, modified, and chemically-modified nucleotide monomers include N^6-methyladenosine nucleotides.

[00238] Examples of non-natural, modified, and chemically-modified nucleotide monomers include nucleotide monomers with modified bases 5-(3-amino)propyluridine, 5-(2-mercapto)ethyluridine, 5-bromouridine; 8-bromoguanosine, or 7-deazaadenosine.

[00239] Examples of non-natural, modified, and chemically-modified nucleotide monomers include 2'-O-aminopropyl substituted nucleotides.

[00240] Examples of non-natural, modified, and chemically-modified nucleotide monomers include replacing the 2'-OH group of a nucleotide with a 2'-R, a 2'-OR, a 2'-halogen, a 2'-SR, or a 2'-amino, where R can be H, alkyl, alkenyl, or alkynyl.

[00241] A guide molecule of this invention, a crRNA, a guide chain, or a tracrRNA may contain any one or more of the non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides shown above.

[00242] In some aspects, a guide compound of this invention can be described by a sequence of attached bases, and being substituted or modified forms thereof. As used herein, substituted or modified forms include differently substituted UNA monomers, as well as differently substituted or modified nucleic acid monomers, as are further described herein.

[00243] Some examples of modified nucleotides are given in Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, 1984.

[00244] U-Guide compounds composed of UNA monomers

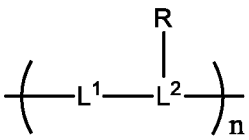
[00245] Aspects of this invention can provide structures and compositions for U-Guide molecules for gene editing that are UNA-monomer containing oligomeric compounds.

[00246] The oligomeric U-Guide agents may incorporate one or more UNA monomers. Oligomeric molecules of this invention can be used as active agents in formulations for gene editing therapeutics.

[00247] In some embodiments, this invention provides oligomeric U-Guide compounds having a structure that incorporates novel combinations of UNA monomers with certain natural nucleotides, non-natural nucleotides, modified nucleotides, or chemically-modified nucleotides.

[00248] In further aspects, the oligomeric U-Guide compounds of this invention can be pharmacologically active molecules. A U-Guide of this invention can be used as an active pharmaceutical ingredient for gene editing.

A U-Guide molecule of this invention can have the structure of Formula I



Formula I

wherein L^1 is a linkage, n is from 39 to 46, and for each occurrence L^2 is a UNA linker group having the formula $–C^1–C^2–C^3–$, where R is attached to C^2 and has the formula

$-OCH(CH_2R^3)R^5$, where R^3 is $—OR^4$, $—SR^4$, $—NR^4{}_2$, $—NH(C=O)R^4$, morpholino, morpholin-1-yl, piperazin-1-yl, or 4-alkanoyl-piperazin-1-yl, where R^4 is the same or different for each occurrence and is H, alkyl, a cholesterol, a lipid molecule, a polyamine, an amino acid, or a polypeptide, and where R^5 is a nucleobase, or $L^2(R)$ is a sugar such as a ribose and R is a nucleobase, or L^2 is a modified sugar such as a modified ribose and R is a nucleobase. In certain embodiments, alkyl is methyl, ethyl, propyl or isopropyl. In certain embodiments, a nucleobase can be a modified nucleobase. L^1 can be a phosphodiester linkage. In further embodiments, $-OCH(CH_2R^3)R^5$ may be $-SCH(CH_2R^3)R^5$, $-CH_2CH(CH_2R^3)R^5$, or $-(SO_2)CH(CH_2R^3)R^5$.

[00249] A U-Guide molecule of this invention can have a guide sequence that is complementary to a target sequence of a genome, where up to three mismatches can occur.

[00250] The target of a U-Guide molecule can be a target nucleic acid. In some embodiments, the target can be any genomic DNA of a subject. A U-Guide molecule can be active for gene editing with a CRISPR/Cas9 system.

[00251] In some aspects, a U-Guide molecule of this invention can have any number of phosphorothioate intermonomer linkages in any position in any strand.

[00252] In some embodiments, any one or more of the intermonomer linkages of a U-Guide molecule can be a phosphodiester, a phosphorothioate including dithioates, a chiral phosphorothioate, and other chemically modified forms.

[00253] For example, the symbol “N” can represent any nucleotide that is complementary to the monomer in the target.

[00254] The symbol “X” in a strand or oligomer represents a UNA monomer. When a UNA monomer appears in a strand of a U-Guide molecule, and is paired with a target, the UNA monomer can have any base attached that would be complementary to the monomer in the target strand.

[00255] When a U-Guide molecule terminates in a UNA monomer, the terminal position has a 1-end, or the terminal position has a 3-end, according to the positional numbering shown above. For example, the U-Guide molecule

SEQ ID NO:1

1-ÛĜCACGGCCACAUUGAUGGCGUUUUAGAGCUAUGCUGUCCÛÛ-3

has a UNA-U monomer 1-end on the left, and a UNA-U monomer 3-end on the right.

[00256] In some embodiments, a U-Guide molecule of this invention can have one or more UNA monomers at the 1-end of the strand, and one or more UNA monomers at the 3-end of the strand.

[00257] In certain embodiments, a U-Guide molecule of this invention may have a length of 39-46 monomers.

[00258] A U-Guide molecule of this invention for editing a gene can have a strand being 39-46 monomers in length, where the monomers can be UNA monomers and nucleic acid monomers.

[00259] A U-Guide molecule can be targeted to a target gene, and can exhibit reduced off-target effects as compared to conventional guide RNAs for CRISP/Cas9 gene editing.

[00260] Off target sites, based on sequence homology to the target, can be determined by constructing an episomally replicated reporter plasmid with either the target or off-target sequence. The reporter can be co-transfected with the U-Guide molecules into mammalian cells. The plasmids can be isolated to perform a T7 endonuclease I assay. Alternatively, sequencing of off-target can be done with PCR using a primer set flanking the potential off-target site.

[00261] A U-Guide molecule can be targeted to a target gene, and can exhibit increased efficiency of gene editing as compared to conventional guide RNAs for CRISP/Cas9 gene editing.

[00262] With a U-Guide molecule of this invention, the average rate of mutation of a genomic target can be at least 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%.

[00263] A U-Guide molecule of this disclosure may comprise naturally-occurring nucleic acid nucleotides, and modifications thereof that are compatible with gene editing activity.

[00264] As used herein, the term strand refers to a single, contiguous chain of monomers, the chain having any number of internal monomers and two end monomers, where each end monomer is attached to one internal monomer on one side, and is not attached to a monomer on the other side, so that it ends the chain.

[00265] The monomers of a U-Guide molecule may be attached via phosphodiester linkages, phosphorothioate linkages, gapped linkages, and other variations.

[00266] In some embodiments, a U-Guide molecule can include mismatches in complementarity between the guide sequence and the target sequence. In further embodiments, a U-Guide molecule may have 1, or 2, or 3 mismatches to the target.

[00267] The target of a U-Guide molecule can be a target nucleic acid of a target gene.

[00268] In certain embodiments, a U-Guide molecule can be a single strand that folds upon itself and hybridizes to itself to form a double stranded region having a connecting loop at one end.

[00269] In some embodiments, an U-Guide molecule of this invention may have a strand being 39-46 monomers in length, where any monomer that is not a UNA monomer can be a Q monomer.

[00270] In some embodiments, an U-Guide molecule of this invention may have a strand being 39-46 monomers in length, where any monomer that is not a UNA monomer can be a Q monomer, and where the number of Q monomers is less than twenty.

[00271] In some embodiments, an U-Guide molecule of this invention may have a strand being 39-46 monomers in length, where any monomer that is not a UNA monomer can be a Q monomer, and where the number of Q monomers is less than twelve.

[00272] In some embodiments, an U-Guide molecule of this invention may have a strand being 39-46 monomers in length, where any monomer that is not a UNA monomer can be a Q monomer, and where the number of Q monomers is less than ten.

[00273] In some embodiments, an U-Guide molecule of this invention may have a strand being 39-46 monomers in length, where any monomer that is not a UNA monomer can be a 2'-O-Methyl modified ribonucleotide.

[00274] Gene Editing

[00275] In some embodiments, the guide molecules of this invention can be used to edit any target portion of a TTR gene, when the target is flanked by a 3' protospacer-adjacent motif (PAM).

[00276] Examples of genes and/or polynucleotides that can be edited with the guide molecules of this invention include TTR, which may be related to amyloid neuropathy and amyloidosis.

[00277] In certain embodiments, this invention further contemplates methods for preventing, treating or ameliorating transthyretin-related hereditary amyloidosis.

[00278] Pharmaceutical compositions

[00279] In some aspects, this invention provides pharmaceutical compositions containing an oligomeric compound and a pharmaceutically acceptable carrier.

[00280] A pharmaceutical composition can be capable of local or systemic administration. In some aspects, a pharmaceutical composition can be capable of any modality of administration. In certain aspects, the administration can be intravenous, subcutaneous, pulmonary, intramuscular, intraperitoneal, dermal, oral, or nasal administration.

[00281] Embodiments of this invention include pharmaceutical compositions containing an oligomeric compound in a lipid formulation.

[00282] In some embodiments, a pharmaceutical composition may comprise one or more lipids selected from cationic lipids, anionic lipids, sterols, pegylated lipids, and any combination of the foregoing.

[00283] In certain embodiments, a pharmaceutical composition can be substantially free of liposomes.

[00284] In further embodiments, a pharmaceutical composition can include liposomes or nanoparticles.

[00285] Some examples of lipids and lipid compositions for delivery of an active molecule of this invention are given in WO/2015/074085, which is hereby incorporated by reference in its entirety.

[00286] In additional embodiments, a pharmaceutical composition can contain an oligomeric compound within a viral or bacterial vector.

[00287] A pharmaceutical composition of this disclosure may include carriers, diluents or excipients as are known in the art. Examples of pharmaceutical compositions are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro ed. 1985).

[00288] Examples of excipients for a pharmaceutical composition include antioxidants, suspending agents, dispersing agents, preservatives, buffering agents, tonicity agents, and surfactants.

[00289] An effective dose of an agent or pharmaceutical formulation of this invention can be an amount that is sufficient to cause gene editing in vivo.

[00290] An effective dose of an agent or pharmaceutical formulation of this invention can be an amount that is sufficient to cause an average rate of mutation of a genomic target in vivo of at least 10%, or at least 15%, or at least 20%, or at least 25%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%.

[00291] A therapeutically effective dose can be an amount of an agent or formulation that is sufficient to cause a therapeutic effect. A therapeutically effective dose can be administered in one or more separate administrations, and by different routes.

[00292] A therapeutically effective dose, upon administration, can result in serum levels of an active agent of 1-1000 pg/ml, or 1-1000 ng/ml, or 1-1000 μg/ml, or more.

[00293] A therapeutically effective dose of an active agent in vivo can be a dose of 0.001-0.01 mg/kg body weight, or 0.01-0.1 mg/kg, or 0.1-1 mg/kg, or 1-10 mg/kg, or 10-100 mg/kg.

[00294] A therapeutically effective dose of an active agent in vivo can be a dose of 0.001 mg/kg body weight, or 0.01 mg/kg, or 0.1 mg/kg, or 1 mg/kg, or 2 mg/kg, or 3 mg/kg, or 4 mg/kg, or 5 mg/kg, or more.

[00295] Autosomal Dominant Diseases

[00296] Examples of diseases and/or conditions for which the guide molecules of this invention can be utilized include those in Table 3.

Table 3: Autosomal Dominant Diseases

Autosomal Dominant Disease	Age / Notes	Related gene
Acropectoral syndrome		
Acute intermittent porphyria	Adulthood. Attacks are treated with either glucose loading or hemin. These are specific treatments that lower the production of heme pathway intermediates by the liver.	HMBS gene
Adermatoglyphia		
Albright's hereditary osteodystrophy		
Arakawa's syndrome II		
Aromatase excess syndrome	~8-18 years old	Mutations in aromatase gene
Autosomal dominant cerebellar ataxia		
Axenfeld syndrome		
Bethlem myopathy		
Birt–Hogg–Dubé syndrome	Liver complications, progressive liver dysfunction, portal hypertension with varices, hypersplenism, and rarely overt liver failure with cirrhosis. Liver cancer.	Unknown, random
Boomerang dysplasia		
Branchio-oto-renal syndrome		
Buschke–Ollendorff syndrome		
Camurati–Engelmann disease	Appears in childhood and is considered to be inherited. The disease is slowly progressive	Mutations in the TGFB1 gene
Central core disease	Reye's syndrome occurs almost exclusively in children. Acute liver failure/coma, death.	Unknown, possible damage to cellular mitochondria
Collagen disease		
Collagenopathy, types II and XI		
Congenital distal spinal muscular atrophy		
Congenital stromal corneal dystrophy		
Costello syndrome		
Currarino syndrome	Birth to 64 years old	Mutation in the HLXB9 homeobox gene

Autosomal Dominant Disease	Age / Notes	Related gene
Darier's disease		
De Vivo disease		
Dentatorubral-pallidoluysian atrophy		
Dermatopathia pigmentosa reticularis		
DiGeorge syndrome		
Dysfibrinogenemia	Adulthood (20's)	Mutation controlling production of liver fibrinogen
Familial atrial fibrillation		
Familial hypercholesterolemia	Inherited condition that causes high levels of LDL cholesterol, beginning at birth, and heart attacks at an early age.	Mutations in APOB, LDLR, LDLRAP1, and PCSK9
Familial male-limited precocious puberty		
Feingold syndrome		
Felty's syndrome	50's, 60's	Unknown
Flynn–Aird syndrome		
Gardner's syndrome	Birth to age 5	Mutations in the APC gene
Gillespie syndrome		
Gray platelet syndrome		
Greig cephalopolysyndactyly syndrome		
Hajdu–Cheney syndrome		
Hawkinsinuria		
Hay–Wells syndrome		
Hereditary elliptocytosis		
Hereditary hemorrhagic telangiectasia	Age-dependent, adolescence or later. Arteriovenous malformation (AVM) is one of the signs/symptoms, predominantly the lungs (50%), liver (30–70%), brain (10%).	Mutations in ACVRL1 gene
Hereditary mucoepithelial dysplasia		
Hereditary spherocytosis	Acute cases can threaten to cause hypoxia through anemia and acute kernicterus through hyperbilirubinemia, particularly in newborns.	Mutations in the ANK1 gene. (also, SPTB, SPTA, SLC4A1, EPB42)
Holt–Oram syndrome		
Hypertrophic cardiomyopathy		
Hypoalphalipoproteinemia		
Jackson–Weiss syndrome		
Keratolytic winter erythema		
Kniest dysplasia		
Kostmann syndrome		
Langer–Giedion syndrome		
Larsen syndrome		
Liddle's syndrome		
Marfan syndrome		

Autosomal Dominant Disease	Age / Notes	Related gene
Marshall syndrome		
Medullary cystic kidney disease		
Metachondromatosis		
Miller–Dieker syndrome		
MOMO syndrome		
Monilethrix		
Multiple endocrine neoplasia		
Multiple endocrine neoplasia type 1		
Multiple endocrine neoplasia type 2		
Multiple endocrine neoplasia type 2b		
Myelokathexis		
Myotonic dystrophy		
Naegeli–Franceschetti–Jadassohn syndrome		
Nail–patella syndrome		
Noonan syndrome		
Oculopharyngeal muscular dystrophy		
Pachyonychia congenita		
Pallister–Hall syndrome		
PAPA syndrome		
Papillorenal syndrome		
Parastremmatic dwarfism		
Pelger–Huet anomaly		
Peutz–Jeghers syndrome	The average age of first diagnosis is 23, but the lesions can be identified at birth by an astute pediatrician	Mutations in the STK11 gene
Piebaldism		
Platyspondylic lethal skeletal dysplasia, Torrance type		
Popliteal pterygium syndrome		
Porphyria cutanea tarda	Late adulthood between the ages of 30 to 40 years.	Inherited mutations in the UROD (20%).
RASopathy		
Reis–Bucklers corneal dystrophy		
Romano–Ward syndrome		
Rosselli–Gulienetti syndrome		
Roussy–Lévy syndrome		
Rubinstein–Taybi syndrome		
Saethre–Chotzen syndrome		
Schmitt Gillenwater Kelly syndrome		
Short QT syndrome		
Singleton Merten syndrome		
Spinal muscular atrophy with lower extremity predominance		
Spinocerebellar ataxia		
Spinocerebellar ataxia type-6		
Spondyloepimetaphyseal dysplasia, Strudwick type		

Autosomal Dominant Disease	Age / Notes	Related gene
Spondyloepiphyseal dysplasia congenita		
Spondyloperipheral dysplasia		
Stickler syndrome		
Tietz syndrome		
Timothy syndrome		
Treacher Collins syndrome		
Tuberous sclerosis	Liver hamartomas. Essentially liver hamartoma embryonic dysplasia and tumor characteristics, from the surgical point of view will continue to hepatic disease classified as benign.	Tuberous Sclerosis, mutation of TSC1 or TSC2
Upington disease		
Variegate porphyria	Liver imaging beginning at age 50 years in those who have experienced persistent elevations in porphobilinogen or porphyrins may detect early hepatocellular carcinoma.	Mutations in the PPOX gene
Vitelliform macular dystrophy		
Von Hippel–Lindau disease		
Von Willebrand disease	Age 5-14 years, age 1-4 years and age 15-29 years. Age 75+ years and age < 1 years rare.	Mutations in the VWF gene
Wallis–Zieff–Goldblatt syndrome		
WHIM syndrome		
White sponge nevus		
Worth syndrome		
Zaspopathy		
Zimmermann–Laband syndrome		
Zori–Stalker–Williams syndrome		

[00297] Protocol for assessment of mTTR gene editing by T7 assay

[00298] Hepa 1-6 cells expressing WT mouse TTR were transfected by LIPOFECTAMINE MESSENGERMAX reagent with Cas9 mRNA 4 hours prior to transfection with the UNA-Guide or comparative guide, each of which was a pre-annealed crRNA:tracrRNA unit targeting exon 2 of mTTR. 48 h following transfection, genomic DNA was isolated and a 459 bp fragment of mTTR was amplified using primers

SEQ ID NO:2

5'CTGGTGCACAGCAGTGCATCT3'

and

SEQ ID NO:3

5'CCTCTCTCTGAGCCCTCTAGCTGGTA3'.

[00299] The PCR product was then heated at 98°C for 5 minutes, and then slowly allowed to cool to room temperature for heteroduplex formation. The T7 endonuclease

assay was then performed to assess gene editing. Image J analysis software was used to determine the percentage of Indels generated using the formula % Indel = 100 x (1-(1-Cleaved DNA fragment Area/Total Area)$^{1/2}$).

[00300] ELISA assessment of secreted mTTR protein knockdown by CRISPR/Cas9 gene editing

[00301] Hepa 1-6 cells expressing WT mouse TTR were transfected by LIPOFECTAMINE MESSENGERMAX reagent with Cas9 mRNA 4 hours prior to transfection with the UNA-Guide or comparative guide, each of which was a pre-annealed crRNA:tracrRNA targeting exon 2 of mTTR. 48 h following transfection, the supernatant was collected and an enzyme-linked immunosorbent assay (ELISA) (mouse prealbumin ELISA kit, Genway) performed to quantify the amount of secreted mouse TTR protein.

[00302] In vivo assessment of gene editing by T7 assay

[00303] Cas9 mRNA and the UNA-Guide or comparative guide, each of which was a pre-annealed crRNA:tracrRNA targeting exon 2 of mTTR, were encapsulated by lipid nanoparticles separately and then mixed together for single administration by tail vein injection at 10 mg/kg total RNA. Six days post-dosing, the female 6-8 week old Balb/c mice were sacrificed and the genomic DNA was isolated and a 459 bp fragment of mTTR amplified using primers

SEQ ID NO:4

5'CTGGTGCACAGCAGTGCATCT3'

and

SEQ ID NO:5

5'CCTCTCTCTGAGCCCTCTAGCTGGTA3'.

[00304] The PCR product was then heated at 98°C for 5 minutes and then slowly allowed to cool to room temperature for heteroduplex formation. The T7 endonuclease assay was then performed to assess gene editing. Image J analysis software was used to determine the percentage of Indels generated using the formula % Indel = 100 x (1-(1-Cleaved DNA fragment Area/Total Area)$^{1/2}$).

[00305] In vivo ELISA assessment of secreted mTTR protein knockdown by CRISPR/Cas9 gene editing

[00306] Cas9 mRNA and the UNA-Guide or comparative guide, each of which was a pre-annealed crRNA:tracrRNA targeting exon 2 of mTTR, were encapsulated by lipid nanoparticles separately and then mixed together for single administration by tail vien injection at 10 mg/kg total RNA. 2, 4 and 6 days post-dosing, serum was collected from the female 6-8 week old Balb/c mice and the amount of secreted mouse TTR protein determined by an enzyme-linked immunosorbent assay (ELISA) (mouse prealbumin ELISA kit, Genway).

[00307] CRISPR/Cas9 gene editing targeting mouse TTR

[00308] A 20-mer guide sequence for V30M mTTR is shown in Table 4.

Table 4: 20-mer guide sequence for V30M mTTR

SEQ ID NO.	SEQUENCE
6	3'-GGA-CGACATCTGCACCGACATTT-5' (V30M mTTR GENE)

[00309] The underlined CAT in Table 4 shows the V30M mutation.

[00310] A U-Guide molecule was synthesized, wherein the molecule contained the 20-mer guide sequence for V30M and a CRISPR sequence of *S. pyogenes*.

[00311] Examples of a 20-mer target length U-Guide molecule for the V30M region of mTTR are shown in Table 5. The molecules in Table 5 contain the target U-Guide attached to a crRNA, as shown in Fig. 2.

Table 5: 20-mer target length U-Guide molecules for editing the V30M region of mTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
7	Ũ*mU*mU*ACAGCCACGUCUACAGCGUUUUAGAGCUAU*mG*mC*mU
8	mU*Ũ*mU*ACAGCCACGUCUACAGCGUUUUAGAGCUAU*mG*mC*mU
9	mU*mU*Ũ*ACAGCCACGUCUACAGCGUUUUAGAGCUAU*mG*mC*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
10	mU*mU*mU*ACAGCCACGUCUACAGCGUUUUAGAGCUAU*mG*mC*Ũ

[00312] In Table 5, N (= A, U, C, G) designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, and Ã, Ũ, Č, Ĝ designate UNA monomers.

EXAMPLES

[00313] **Example 1**: Allele Selective Editing of a TTR genomic site with a U-Guide molecule for CRISPR/Cas9.

[00314] For this experiment, a 357-bp PCR product was generated from human TTR genomic DNA, accession number NC_000018.10, using the primers:

[00315] SEQ ID NO. 11

Forward (intron 1): 5'-tgtcttctctacacccagggcac-3'

[00316] SEQ ID NO. 12

Reverse (exon 2): 5'-gcaaaccacagctagaggagagga-3'.

[00317] Guide sequences of 20-mer length were identified that targeted regions 269-288 and 269-286, respectively, of the human TTR coding region.

[00318] A 20-mer guide sequence for V30M hTTR is shown in Table 6.

Table 6: 20-mer guide sequence for V30M hTTR

SEQ ID NO.	SEQUENCE
13	3'-CGGUAGUUACACCGGUACGU-5' (TARGET GUIDE)
14	5'-CCT-GCCATCAATGTGGCCATGCA-3' (V30M TTR GENE)
15	3'-GGA-CGGTAGTTACACCGGTACGT-5' (V30M TTR GENE)

[00319] In Table 6, the underlined positions show the V30M mutation. In Table 6, SEQ ID NO:13 can also be written in the 5' to 3' direction, and appears in the U-Guide molecules of Table 7 written in the 5' to 3' direction.

[00320] A U-Guide molecule was synthesized, wherein the molecule contained the 20-mer guide sequence for V30M and a CRISPR sequence of *S. pyogenes*.

[00321] Examples of 20-mer target length U-Guide molecules for the V30M region of hTTR are shown in Table 7. The molecules in Table 7 contain the target U-Guide attached to a crRNA, as shown in Fig. 2.

Table 7: 20-mer target length U-Guide molecules for editing the V30M region of hTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
16	ŨGCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUGCU
17	UĜCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUGCU
18	UGČAUGGCCACAUUGAUGGCGUUUUAGAGCUAUGCU
19	UGCÃUGGCCACAUUGAUGGCGUUUUAGAGCUAUGCU
20	UGCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUGCŨ
21	UGCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUGČU
22	UGCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUĜCU
23	UGCAUGGCCACAUUGAUGGCGUUUUAGAGCUAŨGCU
24	ŨmGmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGmCmU
25	mUĜmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGmCmU
26	mUmGČAUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGmCmU
27	mUmGmCÃUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGmCmU
28	mUmGmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGmCŨ
29	mUmGmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUmGČmU
30	mUmGmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAUĜmCmU
31	mUmGmCAUGGCCACAUUGAUGGCGUUUUAGAGCUAŨmGmCmU
32	Ũ*mG*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*mC*mU
33	mU*Ĝ*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*mC*mU
34	mU*mG*Č*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*mC*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
35	mU*mG*mC*ÃUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*mC*mU
36	mU*mG*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*mC*Ũ
37	mU*mG*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*mG*Č*mU
38	mU*mG*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAU*Ĝ*mC*mU
39	mU*mG*mC*AUGGCCACAUUGAUGGCGUUUUAGAGCUAŨ*mG*mC*mU

[00322] In Table 7, N (= A, U, C, G) designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, and Ã, Ũ, Č, Ĝ designate UNA monomers.

[00323] A U-Guide molecule in Table 7 was active for gene editing human TTR. An assay for gene editing human TTR was performed with the 357 bp PCR product. In this assay, the U-Guide molecule is pre-annealed with a tracrRNA to provide the U-Guide/tracr for CRISPR/ Cas9 gene editing.

[00324] In the assay, 293 cells expressing V30M human TTR and 293 cells expressing WT human TTR were each transfected using LIPOFECTAMINE MESSENGER MAX reagent with Cas9 mRNA 4 hours prior to transfection with the U-Guide/tracr. 48 h following transfection, genomic DNA was isolated, and the T7 endonuclease assay performed.

[00325] FIG. 3 shows that using U-Guide molecules UNA1 (SEQ ID NO:32) and UNA2 (SEQ ID NO:35), double strand breaks were made in the 357 bp PCR product to give 275 bp and 82 bp cleavage products.

[00326] The U-Guide molecule SEQ ID NO:32 was surprisingly active for gene editing human TTR with allele selective results. The U-Guide molecule SEQ ID NO:32 showed an extraordinary level of allele selectivity for generating double strand breaks in V30M TTR over wild type TTR.

[00327] As shown in FIG. 4, the U-Guide molecule SEQ ID NO:32 provided 26% editing of V30M TTR, but only about 3% editing of wild type TTR, where the editing

represents the degree of double strand breaks. Thus, the U-Guide molecule SEQ ID NO:32 was surprisingly and extraordinarily active for gene editing human TTR with allele selective results. This example indicates the capability for reduced off target activity.

[00328] The U-Guide molecule SEQ ID NO:35 was surprisingly active for gene editing human TTR with allele selective results. The U-Guide molecule SEQ ID NO:35 showed an extraordinary level of allele selectivity for generating double strand breaks in V30M TTR over wild type TTR.

[00329] As shown in FIG. 4, the U-Guide molecule SEQ ID NO:35 provided 19% editing of V30M TTR, but only about 2% editing of wild type TTR, where the editing represents the degree of double strand breaks. Thus, the U-Guide molecule SEQ ID NO:35 was surprisingly and extraordinarily active for gene editing human TTR with allele selective results. This example indicates the capability for reduced off target activity.

[00330] These results show that the U-Guide molecules of this invention can be used for allele selective gene editing of human TTR. The surprising level of allele selectivity for gene editing of human TTR is shown in FIG. 5. The U-Guide molecule SEQ ID NO:32 provided a high selectivity ratio of 8.7. Further, the U-Guide molecule SEQ ID NO:35 provided a high selectivity ratio of 9.5.

[00331] Further, under the same conditions, a CRISPR/Cas9 guide having the same nucleobase sequence and structure as the U-Guide molecule SEQ ID NOs:32 and 35, but lacking any UNA monomer, had a selectivity ratio of 1.43.

[00332] Assessment of genome editing by sequence trace decomposition was also performed. 293 cells expressing either V30M or WT human TTR were transfected by LIPOFECTAMINE MESSENGERMAX reagent with Cas9 mRNA 4 hours prior to transfection with the comparative guide or UNA-Guide (UNA1), each of which were pre-annealed with tracrRNA, and targeting the V30M mutation of hTTR. 48 h following transfection, genomic DNA was isolated and a 1048 bp fragment of hTTR was amplified. The PCR product was purified and then sanger sequenced.

[00333] The sequencing data files were imported into TIDE (Tracking of Indels by Decomposition) (See, e.g., Brinkman, 2014, Nucl. Acids Res., Vol. 42, No. 22, pp. 1-8) and aligned to the control sequence to determine the relative abundance of aberrant nucleotides following the expected break site to generate the spectrum of insertions and deletions (indels) and their frequencies.

[00334] Fig. 6 shows the indel spectrum for a comparative gRNA guide (non-UNA guide structure) for assessment of genome editing of V30M TTR by sequence trace decomposition (TIDE). The total efficiency was 38.5%.

[00335] Fig. 7 shows the indel spectrum for UNA-guide (UNA1) for assessment of genome editing of V30M TTR by sequence trace decomposition (TIDE). The total efficiency was 33.4%.

[00336] Fig. 8 shows the indel spectrum for a comparative gRNA guide (non-UNA guide structure) for assessment of genome editing of Wild Type TTR by sequence trace decomposition (TIDE). The total efficiency was 26.6%. Thus, the selectivity of the comparative gRNA guide was 38.5/26.6 = 1.4 for V30M TTR over Wild Type TTR.

[00337] Fig. 9 shows the indel spectrum for UNA-guide (UNA1) for assessment of genome editing of Wild Type TTR by sequence trace decomposition (TIDE). The total efficiency was 2.1%. Thus, the selectivity of the UNA-guide (UNA1) was 33.4/2.1 = 15.9 for V30M TTR over Wild Type TTR.

[00338] These results show that the U-Guide molecules of this invention can be used for allele selective gene editing of human TTR. The U-Guide molecules of this invention exhibited a surprisingly high level of allele selectivity for gene editing of human TTR.

[00339] **Example 2**. Allele Selective Editing of a TTR genomic site with a U-Guide molecule for CRISPR/Cas9.

[00340] A 20-mer guide sequence for V30M hTTR is shown in Table 8.

Table 8: 20-mer guide sequence for V30M hTTR

SEQ ID NO.	SEQUENCE

SEQ ID NO.	SEQUENCE
40	3′-AGUUACACCGG**U**ACGUACAC-5′ (TARGET GUIDE)
41	5′-CCA-TCAATGTGGCC**A**TGCATGTG-3′ (V30M TTR GENE)
42	3′-GGT-AGTTACACCGG**T**ACGTACAC-5′ (V30M TTR GENE)

[00341] In Table 8, SEQ ID NO:40 can also be written in the 5' to 3' direction, and appears in the U-Guide molecules of Table 9 written in the 5' to 3' direction.

[00342] As used herein, the term "1 or 5' to 3'" refers to U-Guides having either a UNA monomer on the leftmost end (1 to 3', for example SEQ ID NO:43) or a nucleotide on the leftmost end (5' to 3', for example SEQ ID NO:44).

[00343] A U-Guide molecule was synthesized, wherein the molecule contained the 20-mer guide sequence for V30M and a CRISPR sequence of *S. pyogenes*.

[00344] Examples of 20-mer target length U-Guide molecules for the V30M region of hTTR are shown in Table 9.

Table 9: 20-mer target length U-Guide molecules for editing the V30M region of hTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
43	ČACAUGCAUGGCCACAUUGAGUUUUAGAGCUAUGCU
44	CÃCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUGCU
45	CAČAUGCAUGGCCACAUUGAGUUUUAGAGCUAUGCU
46	CACÃUGCAUGGCCACAUUGAGUUUUAGAGCUAUGCU
47	CACAUGCAUGGCCACAUUGAGUUUUAGAGCUAUGCŨ
48	CACAUGCAUGGCCACAUUGAGUUUUAGAGCUAUGČU
49	CACAUGCAUGGCCACAUUGAGUUUUAGAGCUAUĜCU
50	CACAUGCAUGGCCACAUUGAGUUUUAGAGCUAŨGCU
51	ČmAmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGmCmU
52	mCÃmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGmCmU
53	mCmAČAUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGmCmU
54	mCmAmCÃUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGmCmU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
55	mCmAmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGmCŨ
56	mCmAmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUmGČmU
57	mCmAmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAUĜmCmU
58	mCmAmCAUGCAUGGCCACAUUGAGUUUUAGAGCUAŨmGmCmU
59	Č*mA*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*mC*mU
60	mC*Ã*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*mC*mU
61	mC*mA*Č*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*mC*mU
62	mC*mA*mC*ÃUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*mC*mU
63	mC*mA*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*mC*Ũ
64	mC*mA*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*mG*Č*mU
65	mC*mA*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAU*Ĝ*mC*mU
66	mC*mA*mC*AUGCAUGGCCACAUUGAGUUUUAGAGCUAŨ*mG*mC*mU

[00345] In Table 9, N (= A, U, C, G) designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, and Ã, Ũ, Č, Ĝ designate UNA monomers.

[00346] A U-Guide molecule in Table 9 was active for gene editing human TTR. An assay for gene editing human TTR was performed with the 357 bp PCR product. In this assay, the U-Guide molecule is pre-annealed with a tracrRNA to provide a the U-Guide/tracr for CRISPR/ Cas9 gene editing.

[00347] In the assay, 293 cells expressing V30M human TTR and 293 cells expressing WT human TTR were each transfected using LIPOFECTAMINE MESSENGER MAX reagent with Cas9 mRNA 4 hours prior to transfection with the U-Guide/tracr. 48 h following transfection, genomic DNA was isolated, and the T7 endonuclease assay performed.

[00348] FIG. 10 shows that using U-Guide molecule UNA3 (SEQ ID NO:61), a double strand break was made in the 357 bp PCR product to give 271 bp and 86 bp cleavage products.

[00349] The U-Guide molecule SEQ ID NO:61 was surprisingly active for gene editing human TTR with allele selective results. The U-Guide molecule SEQ ID NO:61 showed an extraordinary level of allele selectivity for generating double strand breaks in V30M TTR over wild type TTR.

[00350] As shown in FIG. 11, the U-Guide molecule SEQ ID NO:61 provided 14% editing of V30M TTR, but only about 3% editing of wild type TTR, where the editing represents the degree of double strand breaks. Thus, the U-Guide molecule SEQ ID NO:62 was surprisingly and extraordinarily active for gene editing human TTR with allele selective results. This example indicates the capability for reduced off target activity.

[00351] These results show that the U-Guide molecules of this invention can be used for allele selective gene editing of human TTR. The surprising level of allele selectivity for gene editing of human TTR is shown in FIG. 12. The U-Guide molecule SEQ ID NO:61 provided a high selectivity ratio of 4.7.

[00352] Thus, the U-Guide molecule SEQ ID NO:62 was extraordinarily active for gene editing human TTR with allele selectivity of V30M TTR over wild type TTR. This example indicates the capability for reduced off target activity.

[00353] **Example 3**: Editing a BIRC5 genomic site with a U-Guide molecule for CRISPR/Cas9.

[00354] Survivin (baculoviral inhibitor of apoptosis repeat-containing 5, human BIRC5, NG_029069.1) can be expressed in tumor cells, especially in breast and lung cancer, and is generally not present in normal cells. Survivin may be an oncogene, and its overexpression in cancer cells may lead to resistance to apoptosis, and increased survival.

[00355] Guide sequences of 20-mer length were identified that targeted certain regions of human BIRC5. The EntreZ Gene ID for these sequences is 332.

[00356] 20-mer guide sequences for BIRC5 are shown in Table 10.

Table 10: 20-mer guide sequences for BIRC5

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
67	GAUGCGGUGGUCCUUGAGAA
68	CAAGAACUGGCCCUUCUUGG
69	GCAGGCGCAGCCCUCCAAGA
70	UUCUGCUUCAAGGAGCUGGA
71	CCAGUUUCAAAAAUUCACCA
72	CAAUAAGAAGAAAGAAUUUG

[00357] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00358] Examples of 20-mer target length U-Guide molecules for BIRC5 are shown in Table 11.

Table 11: 20-mer target length U-Guide molecules for editing BIRC5

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
73	GAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUU
74	ÕGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUÕ
75	ÕĜAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCÕÕ
76	Õ*ĜAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCÕ*Õ
77	mUÕUGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUUÕmU
78	mU*ÕUGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUUÕ*mU
79	mU*Õ*UGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
80	mU*Õ*U*GAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
81	mU*ÕÕGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
82	mU*Õ*ÕGAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
83	mU*Õ*Õ*GAUGCGGUGGUCCUUGAGAAGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
84	CAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUU
85	ÕCAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUÕ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
86	ŨĈAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCŨŨ
87	Ũ*ĈAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
88	mUŨCAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUUŨmU
89	mU*ŨCAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
90	mU*Ũ*CAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
91	mU*Ũ*U*CAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
92	mU*ŨŨCAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
93	mU*Ũ*ŨCAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
94	mU*Ũ*Ũ*CAAGAACUGGCCCUUCUUGGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
95	GCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUU
96	ŨGCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUŨ
97	ŨĜCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCŨŨ
98	Ũ*ĜCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
99	mUŨGCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUUŨmU
100	mU*ŨGCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
101	mU*Ũ*GCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
102	mU*Ũ*U*GCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
103	mU*ŨŨGCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
104	mU*Ũ*ŨGCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
105	mU*Ũ*Ũ*GCAGGCGCAGCCCUCCAAGAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
106	UUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUU
107	ŨUUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUŨ
108	ŨŨUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCŨŨ
109	Ũ*ŨUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
110	mUŨUUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUUŨmU
111	mU*ŨUUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
112	mU*Ũ*UUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
113	mU*Ũ*U*UUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
114	mU*ŨŨUUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
115	mU*Ũ*ŨUUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
116	mU*Ũ*Ũ*UUCUGCUUCAAGGAGCUGGAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
117	CCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUU
118	ŨCCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUŨ
119	ŨĈCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCŨŨ
120	Ũ*ĈCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
121	mUŨCCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUUŨmU
122	mU*ŨCCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
123	mU*Ũ*CCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
124	mU*Ũ*U*CCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
125	mU*ŨŨCCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
126	mU*Ũ*ŨCCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
127	mU*Ũ*Ũ*CCAGUUUCAAAAAUUCACCAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
128	CAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUU
129	ŨCAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUŨ
130	ŨĈAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCŨŨ
131	Ũ*ĈAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
132	mUŨCAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUUŨmU
133	mU*ŨCAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
134	mU*Ũ*CAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
135	mU*Ũ*U*CAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
136	mU*ŨŨCAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
137	mU*Ũ*ŨCAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
138	mU*Ũ*Ũ*CAAUAAGAAGAAAGAAUUUGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00359] In Table 11, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00360] **Example 4**: Editing a CDK16 genomic site with a U-Guide molecule for CRISPR/Cas9.

[00361] The protein encoded by CDK16 belongs to the cdc2/cdkx subfamily of the ser/thr family of protein kinases (human CDK16, NG_012517.1). CDK16 may be associated with in signal transduction cascades in terminally differentiated cells, in exocytosis, and in transport of secretory cargo from the endoplasmic reticulum. Defects and copy-number variants of CDK16 have been associated with various diseases, including intellectual disability and related disorders.

[00362] Guide sequences of 20-mer length were identified that targeted certain regions of human CDK16. The EntreZ Gene ID for these sequences is 5127.

[00363] 20-mer guide sequences for CDK16 are shown in Table 12.

Table 12: 20-mer guide sequences for CDK16

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
139	CGUGCAGAACGAAGUUCCCC
140	UGGAGACUGCACCUCAUCCG
141	UGAUCUCCUUGAGUGCCACA
142	UGAUGUUCCCACAGUCAUCC
143	AGUAGUCCGUGGACCCAAGC
144	CUACCCCAAGUACCGAGCCG

[00364] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00365] Examples of 20-mer target length U-Guide molecules for CDK16 are shown in Table 13.

[00366] Table 13: 20-mer target length U-Guide molecules for editing CDK16

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
145	CGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUU
146	ÜCGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUÜ
147	ÜĈGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCÜÜ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
148	Ũ*ĈGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCŨ*Ũ
149	mUŨCGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUUŨmU
150	mU*ŨCGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUUŨ*mU
151	mU*Ũ*CGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
152	mU*Ũ*U*CGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
153	mU*ŨŨCGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
154	mU*Ũ*ŨCGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
155	mU*Ũ*Ũ*CGUGCAGAACGAAGUUCCCCGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
156	UGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUU
157	ŨUGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUŨ
158	ŨŨGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCŨŨ
159	Ũ*ŨGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
160	mUŨUGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUUŨmU
161	mU*ŨUGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
162	mU*Ũ*UGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
163	mU*Ũ*U*UGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
164	mU*ŨŨUGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
165	mU*Ũ*ŨUGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
166	mU*Ũ*Ũ*UGGAGACUGCACCUCAUCCGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
167	UGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUU
168	ŨUGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUŨ
169	ŨŨGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCŨŨ
170	Ũ*ŨGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
171	mUŨUGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUUŨmU
172	mU*ŨUGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
173	mU*Ũ*UGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
174	mU*Ũ*U*UGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
175	mU*ŨŨUGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
176	mU*Ũ*ŨUGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
177	mU*Ũ*Ũ*UGAUCUCCUUGAGUGCCACAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
178	UGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUU
179	ÕUGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUÕ
180	ÕÕGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCÕÕ
181	Õ*ÕGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCÕ*Õ
182	mUÕUGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUUÕmU
183	mU*ÕUGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUUÕ*mU
184	mU*Õ*UGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
185	mU*Õ*U*UGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
186	mU*ÕÕUGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
187	mU*Õ*ÕUGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
188	mU*Õ*Õ*UGAUGUUCCCACAGUCAUCCGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
189	AGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUU
190	ÕAGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUÕ
191	ÕÃGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCÕÕ
192	Õ*ÃGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCÕ*Õ
193	mUÕAGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUUÕmU
194	mU*ÕAGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUUÕ*mU
195	mU*Õ*AGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
196	mU*Õ*U*AGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
197	mU*ÕÕAGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
198	mU*Õ*ÕAGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
199	mU*Õ*Õ*AGUAGUCCGUGGACCCAAGCGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
200	CUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUU
201	ÕCUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUÕ
202	ÕĈUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCÕÕ
203	Õ*ĈUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCÕ*Õ
204	mUÕCUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUUÕmU
205	mU*ÕCUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUUÕ*mU
206	mU*Õ*CUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
207	mU*Õ*U*CUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
208	mU*ŨŨCUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
209	mU*Ũ*ŨCUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
210	mU*Ũ*Ũ*CUACCCCAAGUACCGAGCCGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00367] In Table 13, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00368] **Example 5**: Editing a STAT3 genomic site with a U-Guide molecule for CRISPR/Cas9.

[00369] Signal transducer and activator of transcription 3 (STAT3) is a transcriptional mediator for many cytokines (human STAT3, NG_007370.1). STAT3 belongs to the family of STAT proteins, which are activated in response to extracellular signaling proteins including the interleukin (IL)-6 family (e.g., IL-5, IL-6, IL-11), among others. STAT3 may be associated in various autoimmune disorders, such as inflammatory bowel disease (IBD), as well as liver disease, gliosis and reactive astrocytes, and other diseases and conditions.

[00370] Guide sequences of 20-mer length were identified that targeted certain regions of human STAT3. The EntreZ Gene ID for these sequences is 6774.

[00371] 20-mer guide sequences for STAT3 are shown in Table 14.

Table 14: 20-mer guide sequences for STAT3

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
211	AGAGCUGAUGGAGCUGCUCC
212	ACUGCUGGUCAAUCUCUCCC
213	CUCUCUUCCGGACAUCCUGA
214	GAGACCGAGGUGUAUCACCA
215	AACCUGGGAUCAAGUGGCCG

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
216	GAAGGUGCUGAACCCUCAGC

[00372] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00373] Examples of 20-mer target length U-Guide molecules for STAT3 are shown in Table 15.

[00374] Table 15: 20-mer target length U-Guide molecules for editing STAT3

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
217	AGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUU
218	ÜAGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUÜ
219	ÜÃGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCÜÜ
220	Ü*ÃGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCÜ*Ü
221	mUÜAGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUUÜmU
222	mU*ÜAGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUUÜ*mU
223	mU*Ü*AGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
224	mU*Ü*U*AGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
225	mU*ÜÜAGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
226	mU*Ü*ÜAGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
227	mU*Ü*Ü*AGAGCUGAUGGAGCUGCUCCGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU
228	ACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUU
229	ÜACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUÜ
230	ÜÃCUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCÜÜ
231	Ü*ÃCUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCÜ*Ü
232	mUÜACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUUÜmU
233	mU*ÜACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUUÜ*mU
234	mU*Ü*ACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
235	mU*Ü*U*ACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
236	mU*ÜÜACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUÜÜ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
237	mU*Ũ*ŨACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
238	mU*Ũ*Ũ*ACUGCUGGUCAAUCUCUCCCGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
239	CUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUU
240	ŨCUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUŨ
241	ŨĈUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCŨŨ
242	Ũ*ĈUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
243	mUŨCUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUUŨmU
244	mU*ŨCUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
245	mU*Ũ*CUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
246	mU*Ũ*U*CUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
247	mU*ŨŨCUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
248	mU*Ũ*ŨCUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
249	mU*Ũ*Ũ*CUCUCUUCCGGACAUCCUGAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
250	GAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUU
251	ŨGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUŨ
252	ŨĜAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCŨŨ
253	Ũ*ĜAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
254	mUŨGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUUŨmU
255	mU*ŨGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
256	mU*Ũ*UGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
257	mU*Ũ*U*GAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
258	mU*ŨŨGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
259	mU*Ũ*ŨGAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
260	mU*Ũ*Ũ*GAGACCGAGGUGUAUCACCAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
261	AACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUU
262	ŨAACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUŨ
263	ŨÃACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCŨŨ
264	Ũ*ÃACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
265	mUŨAACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUUŨmU
266	mU*ŨAACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUUŨ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
267	mU*Ũ*AACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
268	mU*Ũ*U*AACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
269	mU*ŨŨAACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
270	mU*Ũ*ŨAACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
271	mU*Ũ*Ũ*AACCUGGGAUCAAGUGGCCGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
272	GAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUU
273	ŨGAAGGUGCUGAACCCUCAGCAGUUUUAGAGCUAUGCUGUCCUŨ
274	ŨĜAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCŨŨ
275	Ũ*ĜAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCŨ*Ũ
276	mUŨGAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUUŨmU
277	mU*ŨGAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUUŨ*mU
278	mU*Ũ*UGAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
279	mU*Ũ*U*GAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
280	mU*ŨŨGAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
281	mU*Ũ*ŨGAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
282	mU*Ũ*Ũ*GAAGGUGCUGAACCCUCAGCGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00375] In Table 15, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00376] **Example 6**: Editing a CFTR genomic site with a U-Guide molecule for CRISPR/Cas9.

[00377] Cystic fibrosis (CF) is a genetic disorder that substantially affects the respiratory system, causing abnormally thick mucus linings in the lungs. The disease can lead to fatal lung infections, and may also result in various obstructions of the pancreas, hindering digestion. Symptoms of CF include persistent coughing, wheezing or shortness of breath, and an excessive appetite but poor weight gain. Deterioration is inevitable, leading to debility and eventually death. In the United States, the incidence of CF is reported to be 1 in every 3500 births.

[00378] An individual who has the disease inherits a defective cystic fibrosis CFTR gene from each parent. The defective CFTR gene produces the defective protein cystic fibrosis transmembrane conductance regulator, which does not properly regulate the movement of salt and water in and out of cells. The result is thick, sticky mucus in the respiratory, digestive and reproductive systems, as well as increased salt in sweat. There are more than one thousand possible mutations of the CFTR gene.

[00379] Guide sequences of 20-mer length were identified that targeted certain regions of human CFTR (human CFTR, NG_016465.4). The EntreZ Gene ID for these sequences is 1080.

[00380] 20-mer guide sequences for CFTR are shown in Table 16.

Table 16: 20-mer guide sequences for CFTR

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
283	GGUAUAUGUCUGACAAUUCC
284	ACUCCCAGAUUAGCCCCAUG
285	AAGGACAGCCUUCUCUCUAA
286	UGCUGAUCACGCUGAUGCG
287	CUAUUCCCUUUGUCUUGAAG
288	UUCAUUGACAUGCCAACAGA

[00381] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00382] Examples of 20-mer target length U-Guide molecules for CFTR are shown in Table 17.

[00383] Table 17: 20-mer target length U-Guide molecules for editing CFTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
289	GGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUU
290	ÜGGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUÜ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
291	ÕĜGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCÕÕ
292	Õ*ĜGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCÕ*Õ
293	mUÕGGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUUÕmU
294	mU*ÕGGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUUÕ*mU
295	mU*Õ*GGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
296	mU*Õ*U*GGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
297	mU*ÕÕGGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
298	mU*Õ*ÕGGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
299	mU*Õ*Õ*GGUAUAUGUCUGACAAUUCCGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
300	ACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUU
301	ÕACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUÕ
302	ÕÃCUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCÕÕ
303	Õ*ÃCUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCÕ*Õ
304	mUÕACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUUÕmU
305	mU*ÕACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUUÕ*mU
306	mU*Õ*ACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
307	mU*Õ*U*ACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
308	mU*ÕÕACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
309	mU*Õ*ÕACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
310	mU*Õ*Õ*ACUCCCAGAUUAGCCCCAUGGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
311	AAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUU
312	ÕAAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUÕ
313	ÕÃAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCÕÕ
314	Õ*ÃAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCÕ*Õ
315	mUÕAAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUUÕmU
316	mU*ÕAAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUUÕ*mU
317	mU*Õ*AAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
318	mU*Õ*U*AAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
319	mU*ÕÕAAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
320	mU*Õ*ÕAAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
321	mU*Ũ*Ũ*AAGGACAGCCUUCUCUCUAAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
322	UGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUU
323	ŨUGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUŨ
324	ŨŨGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCŨŨ
325	Ũ*ŨGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
326	mUŨUGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUUŨmU
327	mU*ŨUGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
328	mU*Ũ*UGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
329	mU*Ũ*U*UGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
330	mU*ŨŨUGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
331	mU*Ũ*ŨUGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
332	mU*Ũ*Ũ*UGCUGAUCACGCUGAUGCGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
333	CUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUU
334	ŨCUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUŨ
335	ŨĈUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCŨŨ
336	Ũ*ĈUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
337	mUŨCUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUUŨmU
338	mU*ŨCUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
339	mU*Ũ*CUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
340	mU*Ũ*U*CUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
341	mU*ŨŨCUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
342	mU*Ũ*ŨCUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
343	mU*Ũ*Ũ*CUAUUCCCUUUGUCUUGAAGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
344	UUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUU
345	ŨUUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUŨ
346	ŨŨUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCŨŨ
347	Ũ*ŨUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
348	mUŨUUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUUŨmU
349	mU*ŨUUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
350	mU*Ũ*UUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
351	mU*Ũ*U*UUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
352	mU*ŨŨUUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
353	mU*Ũ*ŨUUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
354	mU*Ũ*Ũ*UUCAUUGACAUGCCAACAGAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00384] In Table 17, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00385] **Example** 7: Editing a Factor IX (F9) genomic site with a U-Guide molecule for CRISPR/Cas9.

[00386] Deficiency of Factor IX causes Hemophilia B. There are more than 100 known mutations of Factor IX.

[00387] Guide sequences of 20-mer length were identified that targeted certain regions of human F9 (human F9, NG_007994.1). The EntreZ Gene ID for these sequences is 2158.

[00388] 20-mer guide sequences for F9 are shown in Table 18.

Table 18: 20-mer guide sequences for F9

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
355	CUAAAAGGCAGAUGGUGAUG
356	CUUCCAUACAUUCUCUCUCA
357	AAAGGGACACCAACAUUCAU
358	AAGUCGAUAUCCCUCAGUAC
359	GGUGGAGAAGAUGCCAAACC
360	UUCUGUGCUGGCUUCCAUGA

[00389] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00390] Examples of 20-mer target length U-Guide molecules for F9 are shown in Table 19.

[00391] Table 19: 20-mer target length U-Guide molecules for editing F9

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
361	CUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUU
362	ÕCUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUÕ
363	ÕĈUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCÕÕ
364	Õ*ĈUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCÕ*Õ
365	mUÕCUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUUÕmU
366	mU*ÕCUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUUÕ*mU
367	mU*Õ*CUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
368	mU*Õ*U*CUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
369	mU*ÕÕCUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
370	mU*Õ*ÕCUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
371	mU*Õ*Õ*CUAAAAGGCAGAUGGUGAUGGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
372	CUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUU
373	ÕCUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUÕ
374	ÕĈUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCÕÕ
375	Õ*ĈUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCÕ*Õ
376	mUÕCUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUUÕmU
377	mU*ÕCUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUUÕ*mU
378	mU*Õ*CUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
379	mU*Õ*U*CUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
380	mU*ÕÕCUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
381	mU*Õ*ÕCUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
382	mU*Õ*Õ*CUUCCAUACAUUCUCUCUCAGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
383	AAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUU
384	ÕAAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUÕ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
385	ÕÃAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCÕÕ
386	Õ*ÃAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCÕ*Õ
387	mUÕAAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUUÕmU
388	mU*ÕAAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUUÕ*mU
389	mU*Õ*AAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
390	mU*Õ*U*AAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
391	mU*ÕÕAAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
392	mU*Õ*ÕAAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
393	mU*Õ*Õ*AAAGGGACACCAACAUUCAUGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
394	AAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUU
395	ÕAAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUÕ
396	ÕÃAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCÕÕ
397	Õ*ÃAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCÕ*Õ
398	mUÕAAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUUÕmU
399	mU*ÕAAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUUÕ*mU
400	mU*Õ*AAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
401	mU*Õ*U*AAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
402	mU*ÕÕAAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
403	mU*Õ*ÕAAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
404	mU*Õ*Õ*AAGUCGAUAUCCCUCAGUACGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
405	GGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUU
406	ÕGGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUÕ
407	ÕĜGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCÕÕ
408	Õ*ĜGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCÕ*Õ
409	mUÕGGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUUÕmU
410	mU*ÕGGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUUÕ*mU
411	mU*Õ*GGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
412	mU*Õ*U*GGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
413	mU*ÕÕGGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
414	mU*Õ*ÕGGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
415	mU*Ũ*Ũ*GGUGGAGAAGAUGCCAAACCGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
416	UUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUU
417	ŨUUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUŨ
418	ŨŨUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCŨŨ
419	Ũ*ŨUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCŨ*Ũ
420	mUŨUUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUUŨmU
421	mU*ŨUUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUUŨ*mU
422	mU*Ũ*UUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
423	mU*Ũ*U*UUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
424	mU*ŨŨUUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
425	mU*Ũ*ŨUUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
426	mU*Ũ*Ũ*UUCUGUGCUGGCUUCCAUGAGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00392] In Table 19, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00393] **Example 8**: Editing a KRAS genomic site with a U-Guide molecule for CRISPR/Cas9.

[00394] KRAS protein is essential in normal tissue signaling, and mutation of a KRAS gene is associated with many cancers.

[00395] Guide sequences of 20-mer length were identified that targeted certain regions of human KRAS (human KRAS, NG_007524.1). The EntreZ Gene ID for these sequences is 3845.

[00396] 20-mer guide sequences for KRAS are shown in Table 20.

Table 20: 20-mer guide sequences for KRAS

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
427	CUGAAUUAGCUGUAUCGUCA
428	CAAUGAGGGACCAGUACAUG
429	AGAACAAAUUAAAAGAGUUA
430	AAUCACAUUUAUUUCCUACU
431	UUCUCGAACUAAUGUAUAGA
432	GAAUAUGAUCCAACAAUAGA

[00397] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00398] Examples of 20-mer target length U-Guide molecules for KRAS are shown in Table 21.

[00399] Table 21: 20-mer target length U-Guide molecules for editing KRAS

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
433	CUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUU
434	ÕCUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUÕ
435	ÕĈUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCÕÕ
436	Õ*ĈUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCÕ*Õ
437	mUÕCUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUUÕmU
438	mU*ÕCUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUUÕ*mU
439	mU*Õ*CUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
440	mU*Õ*U*CUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
441	mU*ÕÕCUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
442	mU*Õ*ÕCUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
443	mU*Õ*Õ*CUGAAUUAGCUGUAUCGUCAGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
444	CAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUU
445	ÕCAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUÕ
446	ÕĈAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCÕÕ
447	Õ*ĈAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCÕ*Õ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
448	mUÕCAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUUÕmU
449	mU*ÕCAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUUÕ*mU
450	mU*Õ*CAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
451	mU*Õ*U*CAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
452	mU*ÕÕCAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
453	mU*Õ*ÕCAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
454	mU*Õ*Õ*CAAUGAGGGACCAGUACAUGGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
455	AGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUU
456	ÕAGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUÕ
457	ÕÃGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCÕÕ
458	Õ*ÃGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCÕ*Õ
459	mUÕAGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUUÕmU
460	mU*ÕAGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUUÕ*mU
461	mU*Õ*AGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
462	mU*Õ*U*AGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
463	mU*ÕÕAGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
464	mU*Õ*ÕAGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
465	mU*Õ*Õ*AGAACAAAUUAAAAGAGUUAGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
466	AAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUU
467	ÕAAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUÕ
468	ÕÃAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCÕÕ
469	Õ*ÃAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCÕ*Õ
470	mUÕAAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUUÕmU
471	mU*ÕAAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUUÕ*mU
472	mU*Õ*AAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUU*Õ*mU
473	mU*Õ*U*AAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCU*U*Õ*mU
474	mU*ÕÕAAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUÕÕ*mU
475	mU*Õ*ÕAAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCUÕ*Õ*mU
476	mU*Õ*Õ*AAUCACAUUUAUUUCCUACUGUUUUAGAGCUAUGCUGUCCU*Õ*Õ*mU
477	UUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
478	ÜUUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUÜ
479	ÜÜUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCÜÜ
480	Ü*ÜUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCÜ*Ü
481	mUÜUUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUUÜmU
482	mU*ÜUUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUUÜ*mU
483	mU*Ü*UUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
484	mU*Ü*U*UUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
485	mU*ÜÜUUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
486	mU*Ü*ÜUUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
487	mU*Ü*Ü*UUCUCGAACUAAUGUAUAGAGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU
488	GAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUU
489	ÜGAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUÜ
490	ÜĜAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCÜÜ
491	Ü*ĜAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCÜ*Ü
492	mUÜGAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUUÜmU
493	mU*ÜGAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUUÜ*mU
494	mU*Ü*GAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
495	mU*Ü*U*GAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
496	mU*ÜÜGAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
497	mU*Ü*ÜGAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
498	mU*Ü*Ü*GAAUAUGAUCCAACAAUAGAGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU

[00400] In Table 21, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ü designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00401] **Example 9**: Editing a T cell genomic site with a U-Guide molecule for CRISPR/Cas9.

[00402] A schematic representation of the structure of a chimeric antigen receptor (CAR) is shown in Fig. 13. The CAR is an artificial T cell receptor that is inserted and

expressed in the T cell. ScFv is a single chain fragment variable. V_H is a heavy-chain variable region. V_L is a light-chain variable region. TM is a transmembrane domain. SD is a signaling domain.

[00403] The CAR gene can be inserted into any constitutively expressed gene of a T cell.

[00404] For example, in one embodiment, the CAR gene can be inserted into a CD2 gene (cluster of differentiation 2). CD2 is a cell adhesion molecule found on the surface of T cells, which assists the T cells in adhering to antigen-presenting cells.

[00405] Fig. 14 shows a schematic of a method for introducing a CAR gene into a constitutive CD2 gene of a T cell, in which the CAR is downstream from the CD2. A double strand break is made with a U-Guide molecule of this invention. The gene inserted by homologous recombination can be comprised of a section of CD2, along with P2A and the CAR section. P2A peptide is a self-cleaving peptide that can be used to generate the two separate gene products CD2 protein and CAR protein. The CAR protein receptor can carry the specificity of a mAb against cancer cells of a subject in an adoptive immunotherapy strategy to kill the subject's cancer cells.

[00406] Fig. 15 shows a schematic of a method for introducing a CAR gene into a constitutive CD2 gene of a T cell, in which the CAR is upstream from the CD2.

[00407] Several 20-mer guide sequences for CD2 are shown in Table 22.

Table 22: 20-mer guide sequences for CD2

SEQ ID NO.	SEQUENCE
499	GGGGUACCCCGUCGUCUUUU-5'(U-GUIDE)
500	5'-CCT-CCCCATGGGGCAGCAGAAAA-3'(CD2 GENE)
501	3'-GGA-GGGGTACCCCGTCGTCTTTT-5'(CD2 GENE)
502	AAGACGACCACUUGAACACA-5'(U-GUIDE)
503	5'-CCT-TTCTGCTGGTGAACTTGTGT-3'(CD2 GENE)
504	3'-GGA-AAGACGACCACTTGAACACA-5'(CD2 GENE)

SEQ ID NO.	SEQUENCE
505	GGGGTCTGGAGCTCAAGTCG-5′ (U-GUIDE)
506	5′-CCT-CCCCAGACCTCGAGTTCAGC-3′(CD2 GENE)
507	3′-GGA-GGGGTCTGGAGCTCAAGTCG-5′(CD2 GENE)
508	GAUUAAUUUUUUCUAUCUUU-5′(U-GUIDE)
509	5′-CCT-CTAATTAAAAAAGATAGAAA-3′(CD2 GENE)
510	3′-GGA-GATTAATTTTTTCTATCTTT-5′(CD2 GENE)

[00408] Guide sequences of 20-mer length were identified that targeted certain regions of human CD2.

[00409] 20-mer guide sequences for CD2 are shown in Table 23.

Table 23: 20-mer guide sequences for CD2

SEQ ID NO.	TARGET SEQUENCE 5’ --> 3’
511	UUUUCUGCUGCCCCAUGGGG
512	ACACAAGUUCACCAGCAGAA
513	GCTGAACTCGAGGTCTGGGG
514	UUUCUAUCUUUUUUAAUUAG

[00410] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00411] Examples of 20-mer target length U-Guide molecules for CD2 are shown in Table 24.

[00412] Table 24: 20-mer target length U-Guide molecules for editing CD2

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5’ to 3’)
515	UUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUU
516	ÜUUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUÜ

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
517	ÜÜUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCÜÜ
518	Ü*ÜUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCÜ*Ü
519	mUÜUUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUUÜmU
520	mU*ÜUUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUUÜ*mU
521	mU*Ü*UUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
522	mU*Ü*U*UUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
523	mU*ÜÜUUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
524	mU*Ü*ÜUUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
525	mU*Ü*Ü*UUUUCUGCUGCCCCAUGGGGGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU
526	ACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUU
527	ÜACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUÜ
528	ÜÃCACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCÜÜ
529	Ü*ÃCACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCÜ*Ü
530	mUÜACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUUÜmU
531	mU*ÜACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUUÜ*mU
532	mU*Ü*ACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
533	mU*Ü*U*ACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
534	mU*ÜÜACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
535	mU*Ü*ÜACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
536	mU*Ü*Ü*ACACAAGUUCACCAGCAGAAGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU
537	GCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUU
538	ÜGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUÜ
539	ÜĜCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCÜÜ
540	Ü*ĜCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCÜ*Ü
541	mUÜGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUUÜmU
542	mU*ÜGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUUÜ*mU
543	mU*Ü*UGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
544	mU*Ü*U*GCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
545	mU*ÜÜGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
546	mU*Ü*ÜGCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
547	mU*Ũ*Ũ*GCTGAACTCGAGGTCTGGGGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU
548	UUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUU
549	ŨUUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUŨ
550	ŨŨUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCŨŨ
551	Ũ*ŨUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
552	mUŨUUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUUŨmU
553	mU*ŨUUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
554	mU*Ũ*UUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
555	mU*Ũ*U*UUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
556	mU*ŨŨUUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
557	mU*Ũ*ŨUUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
558	mU*Ũ*Ũ*UUUCUAUCUUUUUUAAUUAGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00413] In Table 24, N designates an RNA monomer, mN designates a 2'-O-methyl-RNA monomer, * designates a 3'-phosphorothioate linkage, Ũ designates a UNA-U monomer, and Ĝ designates a UNA-G monomer.

[00414] **Example 10**: Protocol for sequence trace decomposition (TIDE).

[00415] 293 cells expressing either V30M or WT human TTR were transfected by LIPOFECTAMINE MESSENGERMAX reagent with Cas9 mRNA 4 hours prior to transfection with the comparative guide or UNA-Guide (UNA1), each of which were pre-annealed with tracrRNA, and targeting the V30M mutation of hTTR. 48 h following transfection, genomic DNA was isolated and a 1048 bp fragment of hTTR was amplified using primers

SEQ ID NO:559

5'ACAACTGGTAAGAAGGAGTGAC3' and

SEQ ID NO:560

5'CCTTGGGTTTTGGGTGATCC3'.

[00416] The PCR product was purified and then sanger sequenced using either the SEQ ID NO:561
5'TCGACACTTACGTTCCTGAT3' or
SEQ ID NO:562
5'CATACTTGACCTCTGCCTAC3' primers.

[00417] **Example 11**: Editing a TTR genomic site with a U-Guide molecule for CRISPR/Cas9.

[00418] Guide sequences of 20-mer length were identified that targeted certain regions of human TTR, accession number NC_000018.10.

[00419] 20-mer guide sequences for hTTR are shown in Table 25.

Table 25: 20-mer guide sequences for hTTR

SEQ ID NO.	TARGET SEQUENCE 5' --> 3'
563	TAAGGTGGTGCCGACAGTAG-5' (GUIDE – V122I)
564	5'-CCT-ATTCCACCACGGCTGTCATC-3' (V122I TTR GENE)
565	3'-GGA-TAAGGTGGTGCCGACAGTAG-5' (V122I TTR GENE)
566	GTCAACACTCGGGTACGCCG-5' (GUIDE – L55P)
567	5'-CCT-CAGTTGTGAGCCCATGCGGC-3' (L55P TTR GENE)
568	3'-GGA-GTCAACACTCGGGTACGCCG-5' (L55P TTR GENE)
569	GTCTGTGTTTATGGTCAGGT-5' (GUIDE)
570	5'-CCT-CAGACACAAATACCAGTCCA-3' (SP TTR GENE)
571	3'-GGA-GTCTGTGTTTATGGTCAGGT-5' (SP TTR GENE)

[00420] A U-Guide molecule is synthesized, wherein the molecule contains the 20-mer target sequence and a CRISPR sequence of *S. pyogenes*.

[00421] Examples of 20-mer target length U-Guide molecules for V122I hTTR are shown in Table 26.

Table 26: 20-mer target length U-Guide molecules for editing the V122I region of hTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
572	GATGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUU
573	ÜGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUÜ
574	ÜĜAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCÜÜ
575	Ü*ĜAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCÜ*Ü
576	mUÜUGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUUÜmU
577	mU*ÜUGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUUÜ*mU
578	mU*Ü*UGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
579	mU*Ü*U*GAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
580	mU*ÜÜGGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
581	mU*Ü*ÜGAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU
582	mU*Ü*Ü*GAUGACAGCCGUGGUGGAAUGUUUUAGAGCUAUGCUGUCCU*Ü*Ü*mU

[00422] Examples of 20-mer target length U-Guide molecules for region L55P of hTTR are shown in Table 27.

Table 27: 20-mer target length U-Guide molecules for editing the L55P region of hTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
583	GCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUU
584	ÜGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUÜ
585	ÜĜCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCÜÜ
586	Ü*ĜCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCÜ*Ü
587	mUÜUGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUUÜmU
588	mU*ÜUGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUUÜ*mU
589	mU*Ü*UGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUU*Ü*mU
590	mU*Ü*U*GCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCU*U*Ü*mU
591	mU*ÜÜGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUÜÜ*mU
592	mU*Ü*ÜGCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCUÜ*Ü*mU

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
593	mU*Ũ*Ũ*GCCGCAUGGGCUCACAACUGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00423] Examples of 20-mer target length U-Guide molecules for region SP of hTTR are shown in Table 28.

Table 28: 20-mer target length U-Guide molecules for editing the SP region of hTTR

SEQ ID NO.	U-GUIDE STRUCTURE (1 or 5' to 3')
594	UGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUU
595	ŨUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUŨ
596	ŨŨGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCŨŨ
597	Ũ*ŨGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCŨ*Ũ
598	mUŨUUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUUŨmU
599	mU*ŨUUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUUŨ*mU
600	mU*Ũ*UUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUU*Ũ*mU
601	mU*Ũ*U*UGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCU*U*Ũ*mU
602	mU*ŨŨUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUŨŨ*mU
603	mU*Ũ*ŨUGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCUŨ*Ũ*mU
604	mU*Ũ*Ũ*UGGACUGGUAUUUGUGUCUGGUUUUAGAGCUAUGCUGUCCU*Ũ*Ũ*mU

[00424] **Example 12**: An example of a crRNA for a U-Guide molecule for CRISPR/Cas gene editing is

SEQ ID NO:605

5'-GUUUUAGAGCUAUGCU-3'.

[00425] **Example 13**: An example of a tracrRNA, as used above, for a U-Guide system for CRISPR/Cas gene editing is:

SEQ ID NO:606

5'-mA*mG*mC*mAmUmAmGmCmAAGUUAAAAUAAGGCUAGUCCGUUAUCAAmCmUmUm

GmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU-3'.

[00426] It is understood that this invention is not limited to the particular methodology, protocols, materials, and reagents described, as these may 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 limit the scope of the present invention, which will be encompassed by the appended claims.

[00427] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. As well, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms “comprises,” "comprising", “containing,” "including", and "having" can be used interchangeably.

[00428] Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

[00429] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose.

WHAT IS CLAIMED IS:

1. A guide compound targeted to a genomic DNA, comprising a target guide chain of 14-24 contiguous monomers attached to a crRNA, wherein the guide compound directs CRISPR gene editing of the genomic DNA.

2. The guide compound of claim 1, wherein the guide compound directs double strand breaks in human gene TTR and the target guide chain comprises 16-20 contiguous monomers of 5'-UGCAUGGCCACAUUGAUGGC-3' (SEQ ID NO:13), wherein the crRNA is attached at the 3' end of the target guide chain, and substituted or modified forms thereof.

3. The guide compound of claim 2, wherein the guide compound comprises SEQ ID NO:32.

4. The guide compound of claim 1, wherein the guide compound directs double strand breaks in human gene TTR and the target guide chain comprises 16-20 contiguous monomers of 5'-CACAUGCAUGGCCACAUUGA-3' (SEQ ID NO:40), wherein the crRNA is attached at the 3' end of the target guide chain, and substituted or modified forms thereof.

5. The guide compound of claim 4, wherein the guide compound comprises SEQ ID NO:61.

6. The guide compound of claim 1, wherein the crRNA is 5'-GUUUUAGAGCUAUGCU-3' (SEQ ID NO:605), and substituted or modified forms thereof.

7. The guide compound of claim 1, wherein the monomers comprise UNA monomers and nucleic acid monomers, and wherein the guide compound comprises a sequence of bases targeted to direct CRISPR gene editing of the genomic DNA.

8. The guide compound of claim 2, comprising one or more UNA monomers.

9. The guide compound of claim 4, comprising one or more UNA monomers.

10. The guide compound of claim 7, wherein the sequence of bases of the target guide chain has up to three mismatches from the genomic DNA.

11. The guide compound of claim 7, wherein the guide compound contains one to five UNA monomers.

12. The guide compound of claim 7, wherein the nucleic acid monomers are selected from natural nucleotides, non-natural nucleotides, modified nucleotides, chemically-modified nucleotides, and combinations thereof.

13. The guide compound of claim 7, wherein one or more of the nucleic acid monomers is a 2'-O-methyl ribonucleotide, a 2'-O-methyl purine nucleotide, a 2'-deoxy-2'-fluoro ribonucleotide, a 2'-deoxy-2'-fluoro pyrimidine nucleotide, a 2'-deoxy ribonucleotide, a 2'-deoxy purine nucleotide, a universal base nucleotide, a 5-C-methyl-nucleotide, an inverted deoxyabasic monomer residue, a 3'-end stabilized nucleotide, a 3'-glyceryl nucleotide, a 3'-inverted abasic nucleotide, a 3'-inverted thymidine, a locked nucleic acid nucleotide (LNA), a 2'-O,4'-C-methylene-(D-ribofuranosyl) nucleotide, a 2'-methoxyethoxy (MOE) nucleotide, a 2'-methyl-thio-ethyl, 2'-deoxy-2'-fluoro nucleotide, a 2'-O-methyl nucleotide, a 2′,4′-Constrained 2′-O-Methoxyethyl (cMOE), a 2′-O-Ethyl (cEt), a 2'-amino nucleotide, a 2'-O-amino nucleotide, a 2'-C-allyl nucleotides, a 2'-O-allyl nucleotide, a N^6-methyladenosine nucleotide, a nucleotide with modified base 5-(3-amino)propyluridine, a nucleotide with modified base 5-(2-mercapto)ethyluridine, a nucleotide with modified base 5-bromouridine, a nucleotide with modified base 8-bromoguanosine, a nucleotide with modified base 7-deazaadenosine, a 2'-O-aminopropyl substituted nucleotide, or a nucleotide with a 2'-OH group replaced with a 2'-R, a 2'-OR, a 2'-halogen, a 2'-SR, or a 2'-amino, where R can be H, alkyl, alkenyl, or alkynyl.

14. The guide compound of claim 7, wherein one or more of the last three monomers at each end of the guide compound is connected by a phosphorothioate, a chiral phosphorothioate, or a phosphorodithioate linkage.

15. The guide compound of claim 1, wherein the guide compound directs double strand breaks in a gene selected from TTR, BIRC5, CDK16, STAT3, CFTR, F9, KRAS, and CAR.

16. The guide compound of claim 1, wherein the genomic DNA contains a target disease-related single nucleotide polymorphism.

17. The guide compound of claim 1, wherein the guide compound directs double strand breaks in a disease-related allele.

18. The guide compound of claim 1, wherein the guide compound directs double strand breaks in a disease-related allele selected from V30M TTR, G284R ColA1, L132P Keratin12, R135T Keratin12, G85R SOD1, G272V Tau, P301L Tau, V337M Tau, R406W Tau, Q39STOP beta-Globin, T8993G/C mtDNA, G719S EGFR, and G12C Kras.

19. The guide compound of claim 1, comprising 30-300 contiguous monomers.

20. The guide compound of claim 1, wherein the CRISPR gene editing uses Cas9.

21. The guide compound of claim 7, wherein the guide compound directs gene editing with reduced off target activity.

22. The guide compound of claim 7, wherein the guide compound directs more double strand breaks in a disease-related allele than in the same allele as a wild type.

23. A guide compound of any one of claims 1-22 annealed with a tracrRNA.

24. The guide compound of claim 23, wherein the tracrRNA is derived from *S. pneumonia*, *S. pyogenes*, *N. menigiditis,* or *S. thermophiles*.

25. The guide compound of claim 23, wherein the tracrRNA is SEQ ID NO:606.

26. A guide compound of any one of claims 1-22 annealed with a tracrRNA and complexed with a CRISPR-associated gene editing protein.

27. The guide compound of claim 26, wherein the CRISPR-associated gene editing protein is Cas9.

28. A guide compound targeted to a genomic DNA, wherein the guide compound is a chain of monomers and directs CRISPR gene editing of the genomic DNA, the guide compound comprising a target guide chain, a CRISPR crRNA, and a CRISPR tracrRNA as a single strand, wherein the target guide chain is 14-24 contiguous monomers in length, wherein the monomers comprise UNA monomers and nucleic acid monomers, and wherein the guide compound comprises a sequence of bases targeted to direct CRISPR gene editing of the genomic DNA.

29. The guide compound of claim 28, wherein the guide compound directs gene editing in a CRISPR/Cas9 complex.

30. A pharmaceutical composition comprising one or more guide compounds of claim 23 and a pharmaceutically acceptable carrier.

31. The composition of claim 30, wherein the pharmaceutically acceptable carrier comprises a viral vector or a non-viral vector.

32. The composition of claim 30, wherein the pharmaceutically acceptable carrier comprises liposomes.

33. A method for editing a genomic DNA in a cell, wherein the cell comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising contacting the cell with a composition according to claim 30.

34. The method of claim 33, wherein the editing is disrupting the DNA or repressing transcription of the DNA.

35. The method of claim 33, wherein the editing is achieved with reduced off target activity.

36. The method of claim 33, wherein the CRISPR gene editing enzyme is co-transfected with the composition.

37. A method for editing a genomic DNA in a subject in vivo, wherein the subject comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising administering to the subject a composition according to claim 30.

38. The method of claim 37, wherein the editing is disrupting the DNA or repressing transcription of the DNA.

39. The method of claim 37, wherein the editing is achieved with reduced off target activity.

40. The method of claim 37, wherein the CRISPR gene editing enzyme is co-transfected with the composition.

41. A method for preventing, treating or ameliorating a disease associated with a target genomic DNA in a subject in need, wherein the subject comprises an inducible or constitutive CRISPR gene editing enzyme, the method comprising administering to the subject a composition according to claim 30.

42. The use of a composition of claim 30 for preventing, ameliorating or treating a disease or condition in a subject in need.

43. A composition of claim 30 for use in medical therapy.

44. A composition of claim 30 for use in the treatment of the human or animal body.

45. The use of a composition of claim 30 for preparing or manufacturing a medicament for preventing, ameliorating or treating a disease or condition in a subject in need.

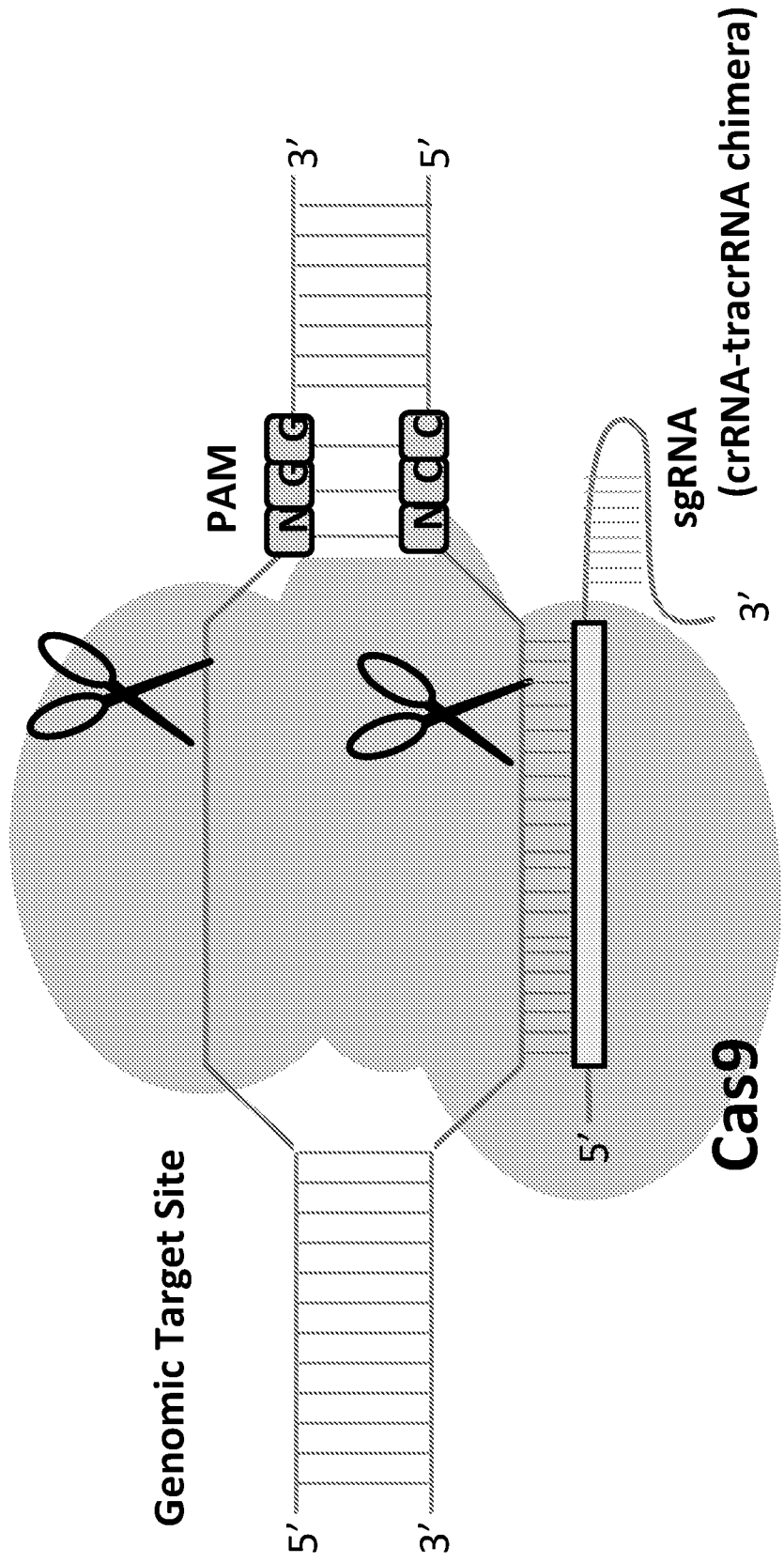

Genomic Target Site
5'
3'
PAM
NGG
NGG
3'
5'
sgRNA
(crRNA-tracrRNA chimera)
3'
5'
Cas9

FIG. 1

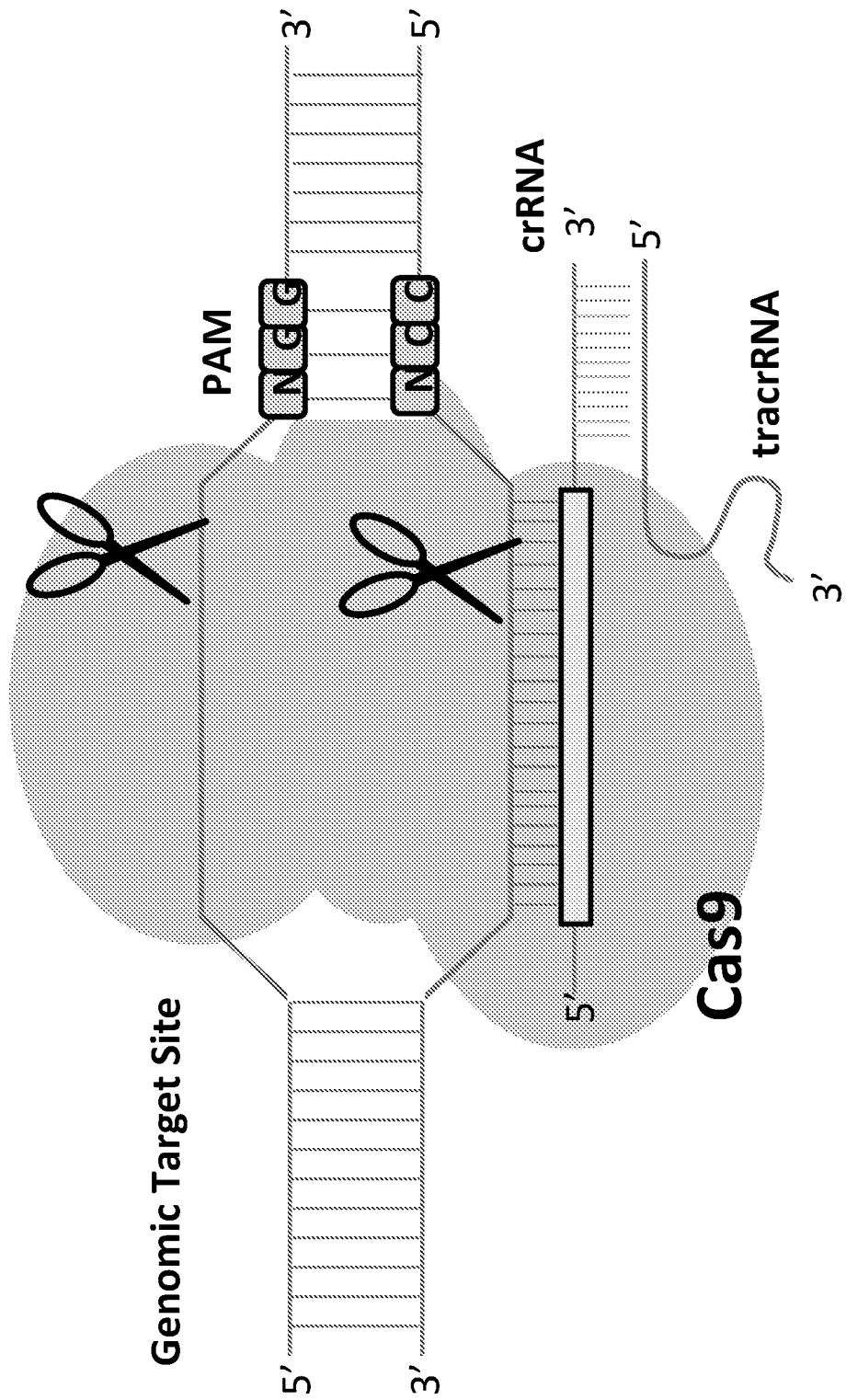

3'
5'
PAM
NGG
NGG
crRNA
3'
5'
tracrRNA
3'
Genomic Target Site
5'
3'
5'
Cas9

FIG. 2

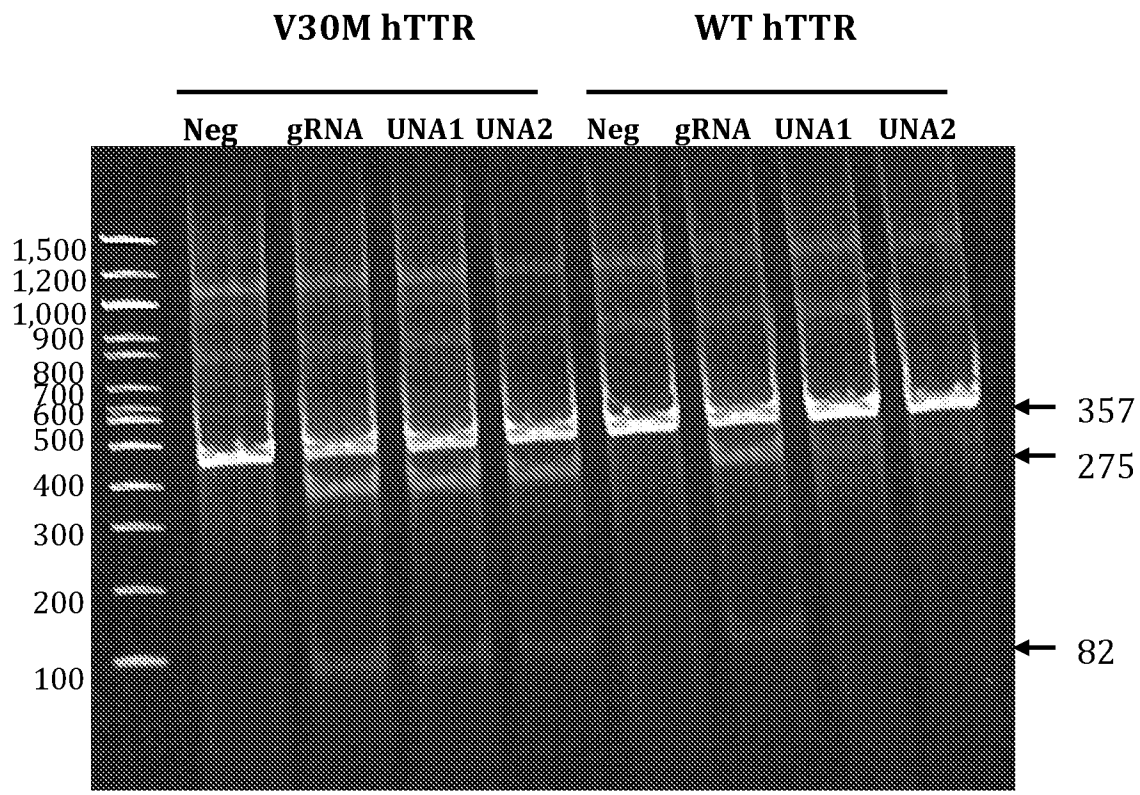

V30M hTTR
WT hTTR
Neg
gRNA
UNA1
UNA2
Neg
gRNA
UNA1
UNA2
1,500
1,200
1,000
900
800
700
600
500
400
300
200
100
357
275
82

FIG. 3

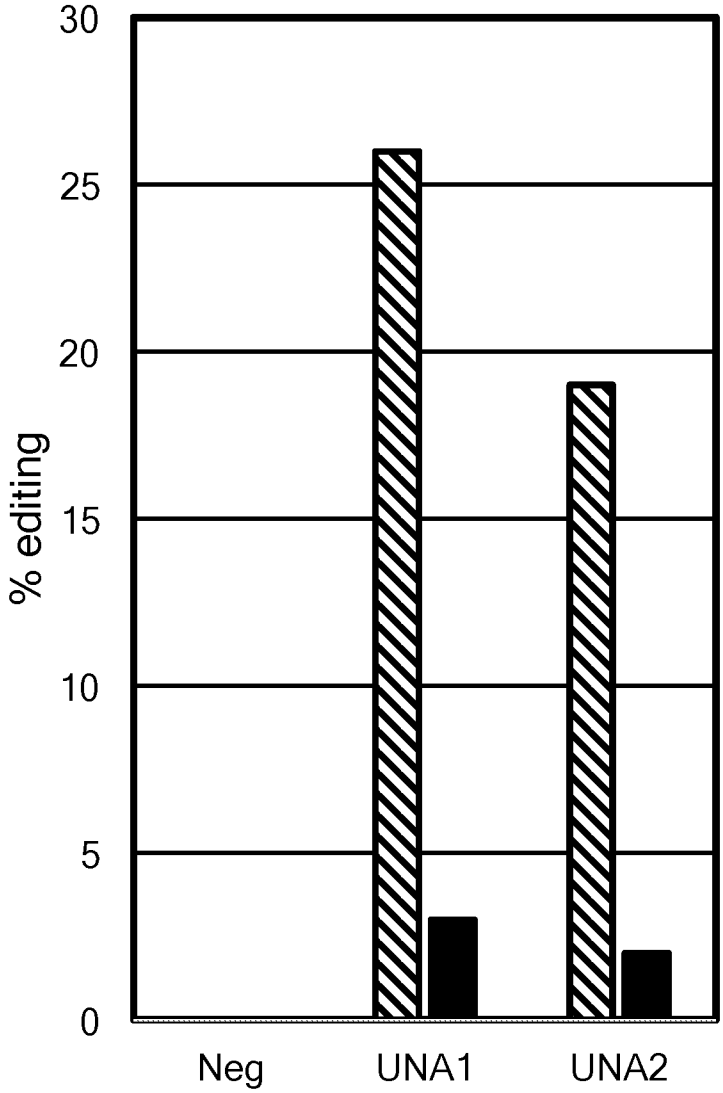


FIG. 4

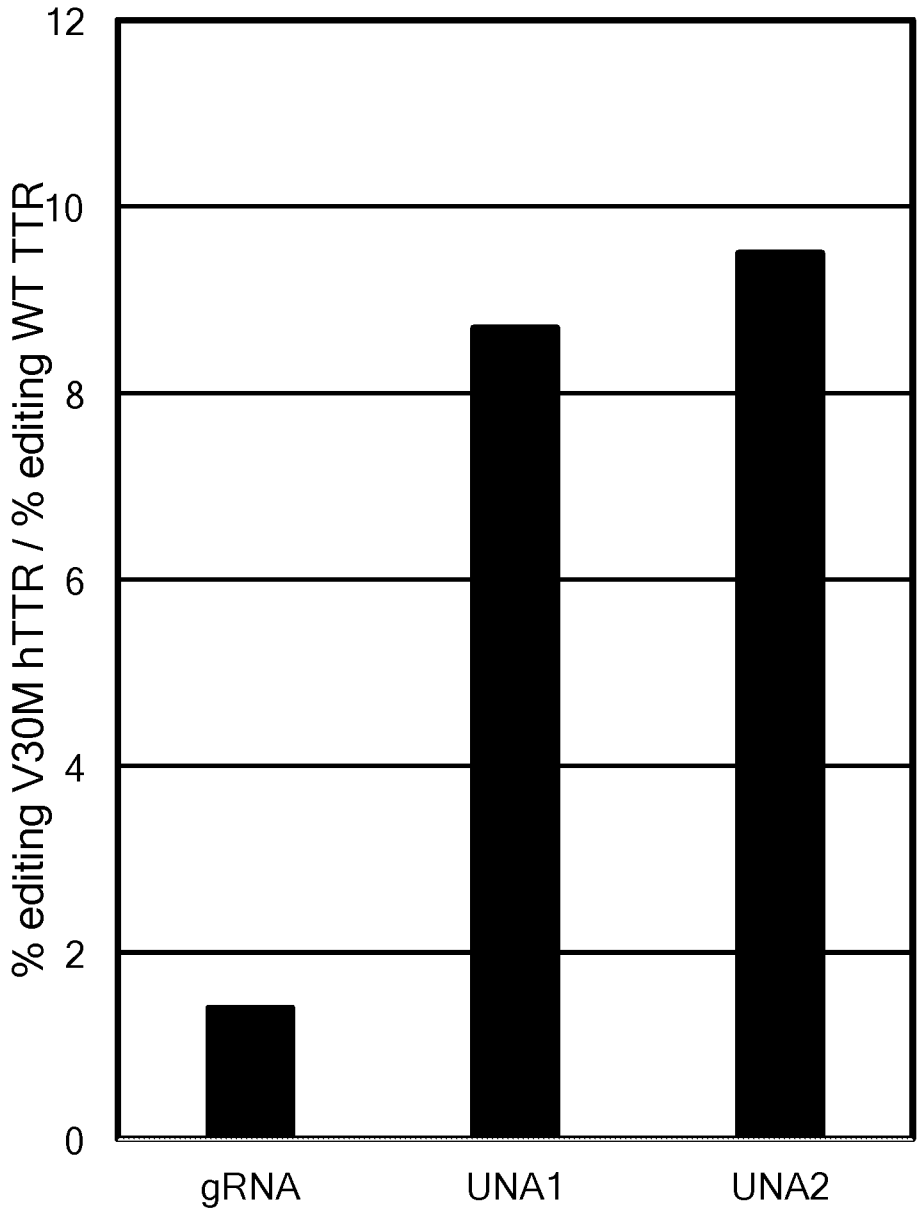


FIG. 5

FIG. 6

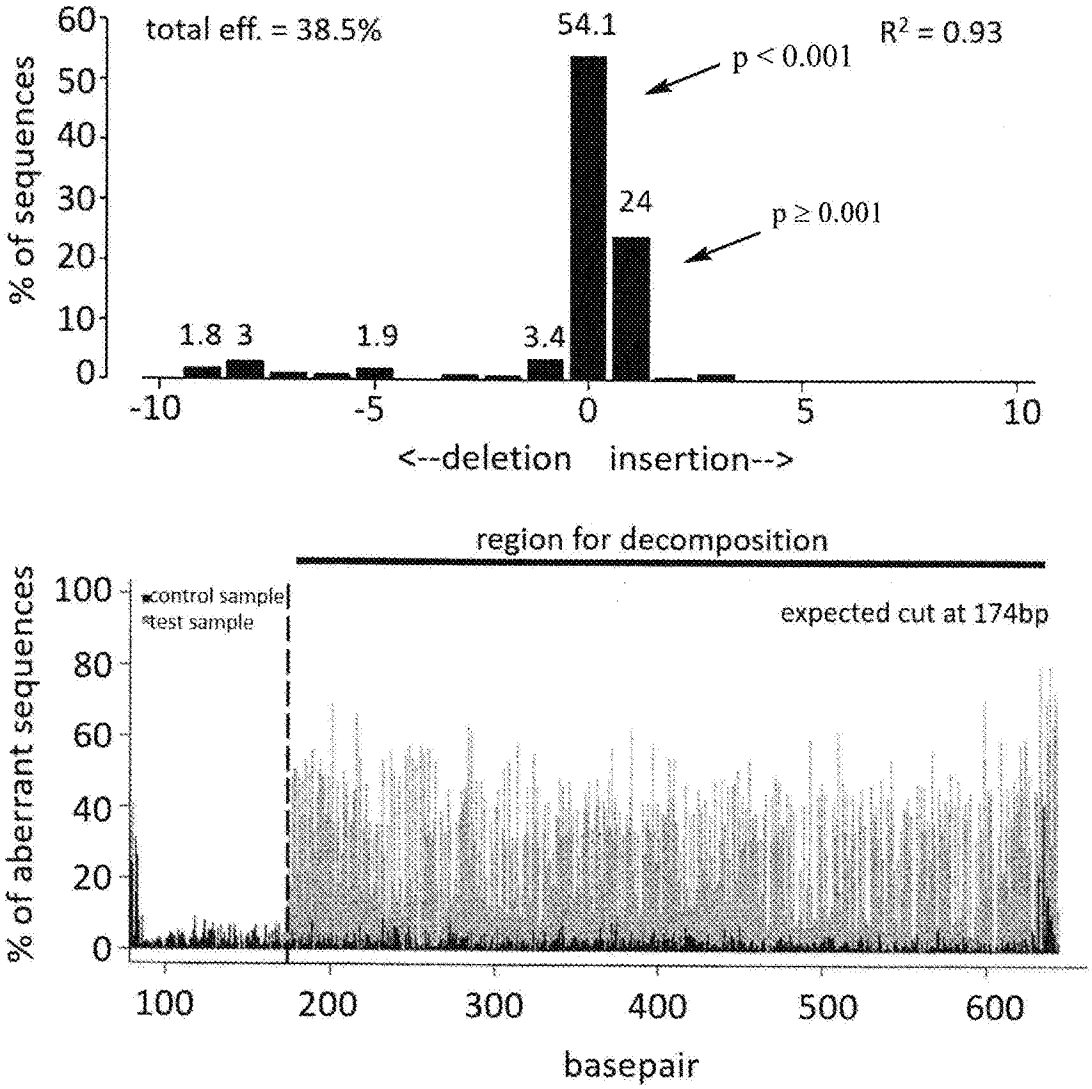

total eff. = 38.5%
54.1
R^2 = 0.93
p < 0.001
p ≥ 0.001
24
1.8
3
1.9
3.4
% of sequences
60
50
40
30
20
10
0
-10
-5
0
5
10
<--deletion insertion-->
region for decomposition
control sample
test sample
expected cut at 174bp
% of aberrant sequences
100
80
60
40
20
0
100
200
300
400
500
600
basepair

FIG. 7

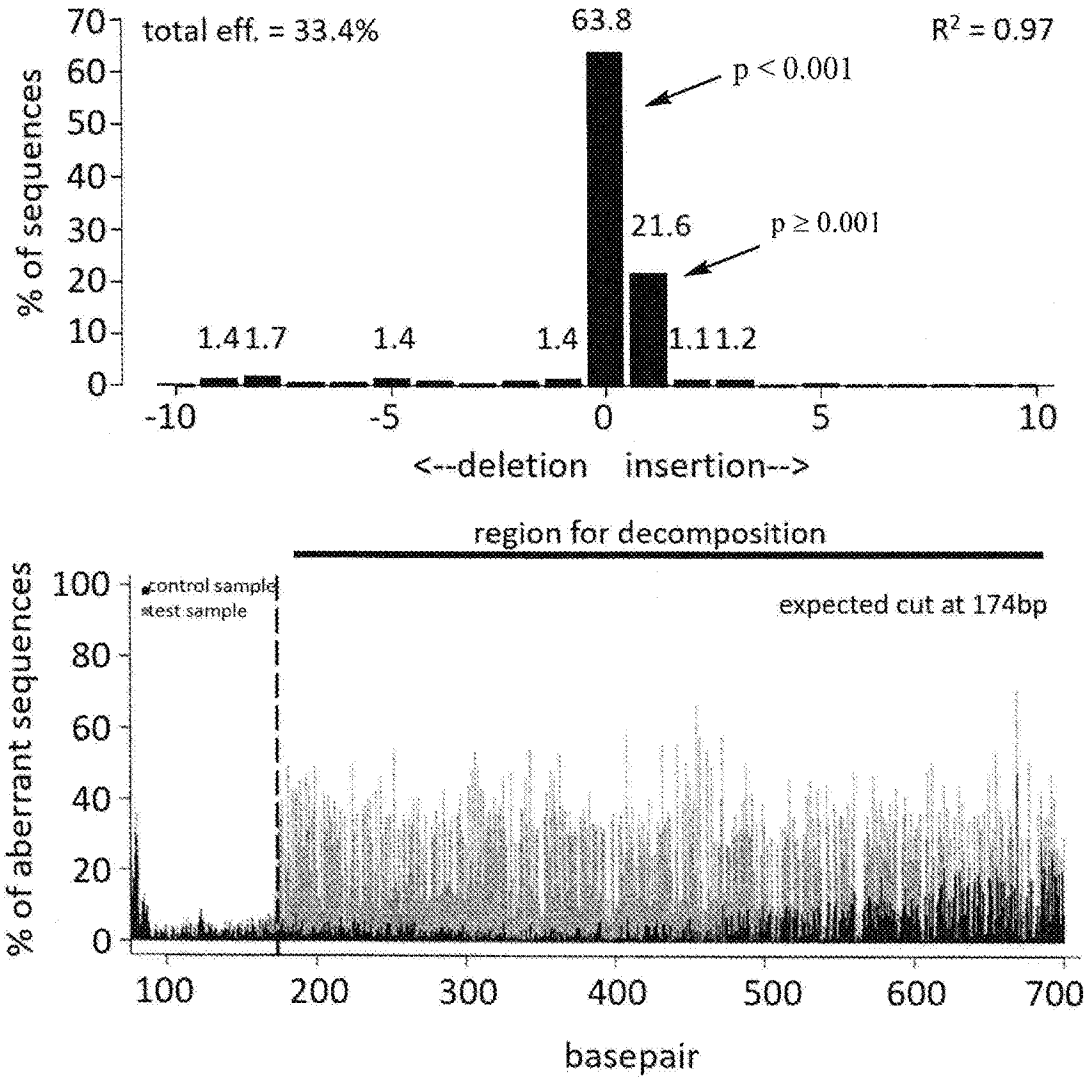

total eff. = 33.4%
R² = 0.97
63.8
p < 0.001
21.6
p ≥ 0.001
1.4 1.7
1.4
1.4
1.1 1.2
% of sequences
<--deletion insertion-->
region for decomposition
control sample
test sample
expected cut at 174bp
% of aberrant sequences
basepair

FIG. 8

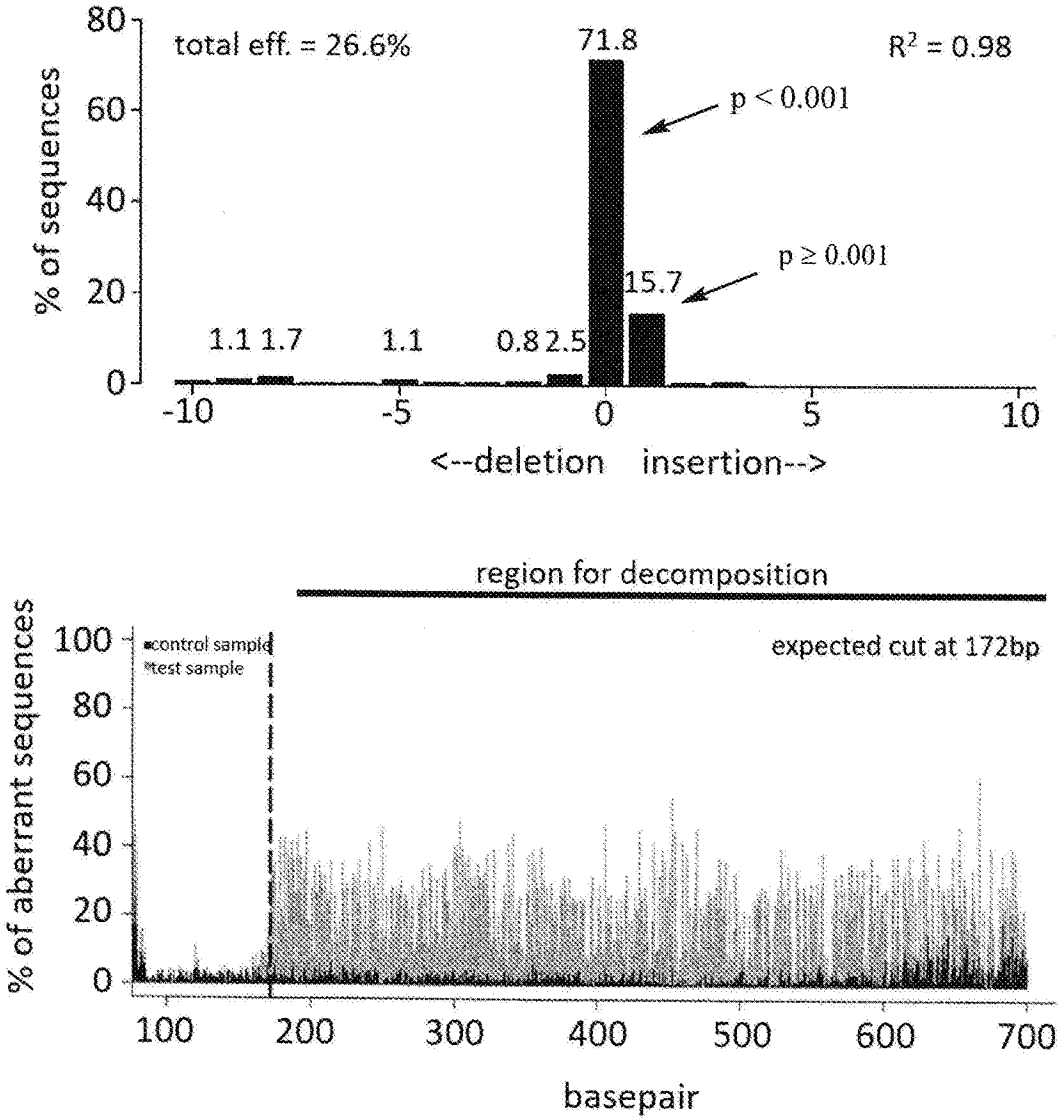
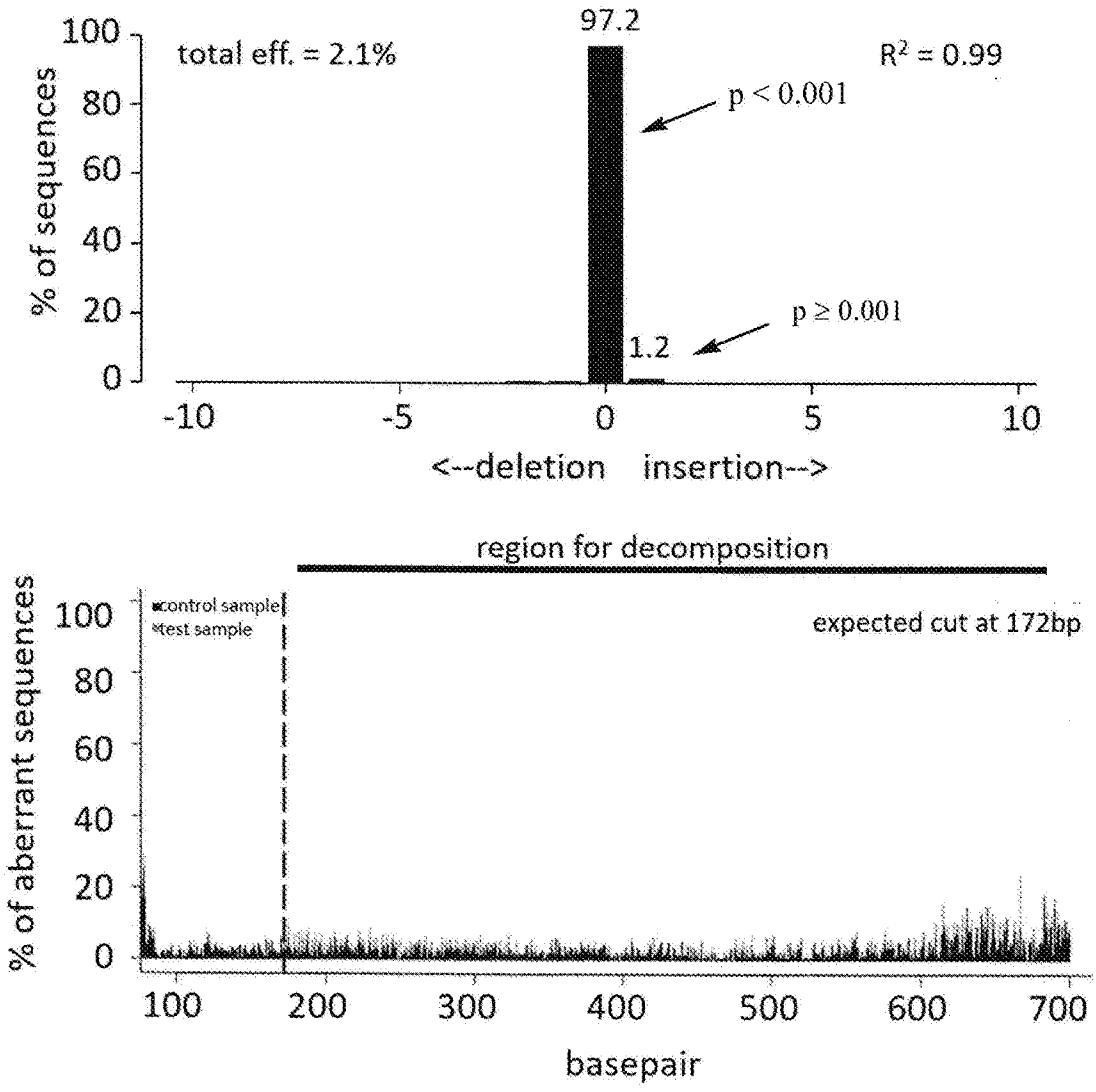

total eff. = 26.6%
71.8
R² = 0.98
p < 0.001
p ≥ 0.001
15.7
1.1 1.7
1.1
0.8 2.5
% of sequences
80
60
40
20
0
-10
-5
0
5
10
<--deletion insertion-->
region for decomposition
control sample
test sample
expected cut at 172bp
% of aberrant sequences
100
80
60
40
20
0
100
200
300
400
500
600
700
basepair

FIG. 9


97.2
total eff. = 2.1%
R^2 = 0.99
p < 0.001
p ≥ 0.001
1.2
% of sequences
100
80
60
40
20
0
-10
-5
0
5
10
<--deletion insertion-->
region for decomposition
control sample
test sample
expected cut at 172bp
% of aberrant sequences
100
80
60
40
20
0
100
200
300
400
500
600
700
basepair

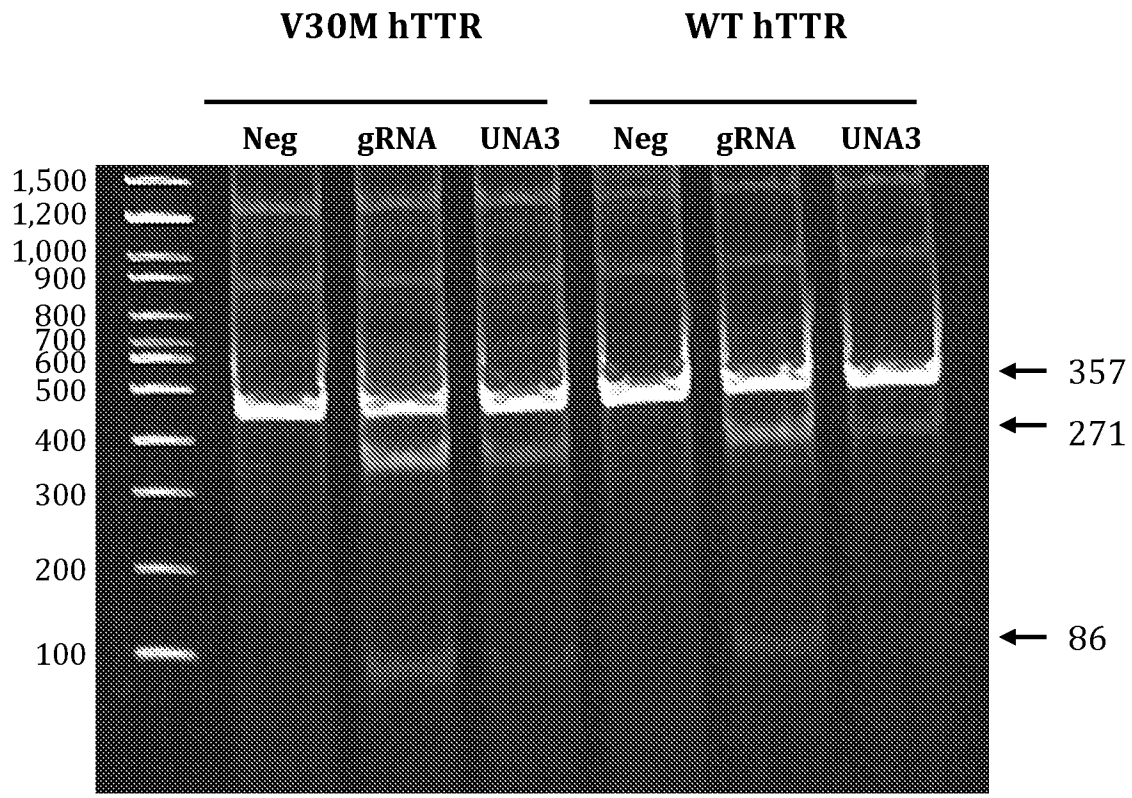

V30M hTTR
WT hTTR
Neg
gRNA
UNA3
Neg
gRNA
UNA3
1,500
1,200
1,000
900
800
700
600
500
400
300
200
100
357
271
86

FIG. 10

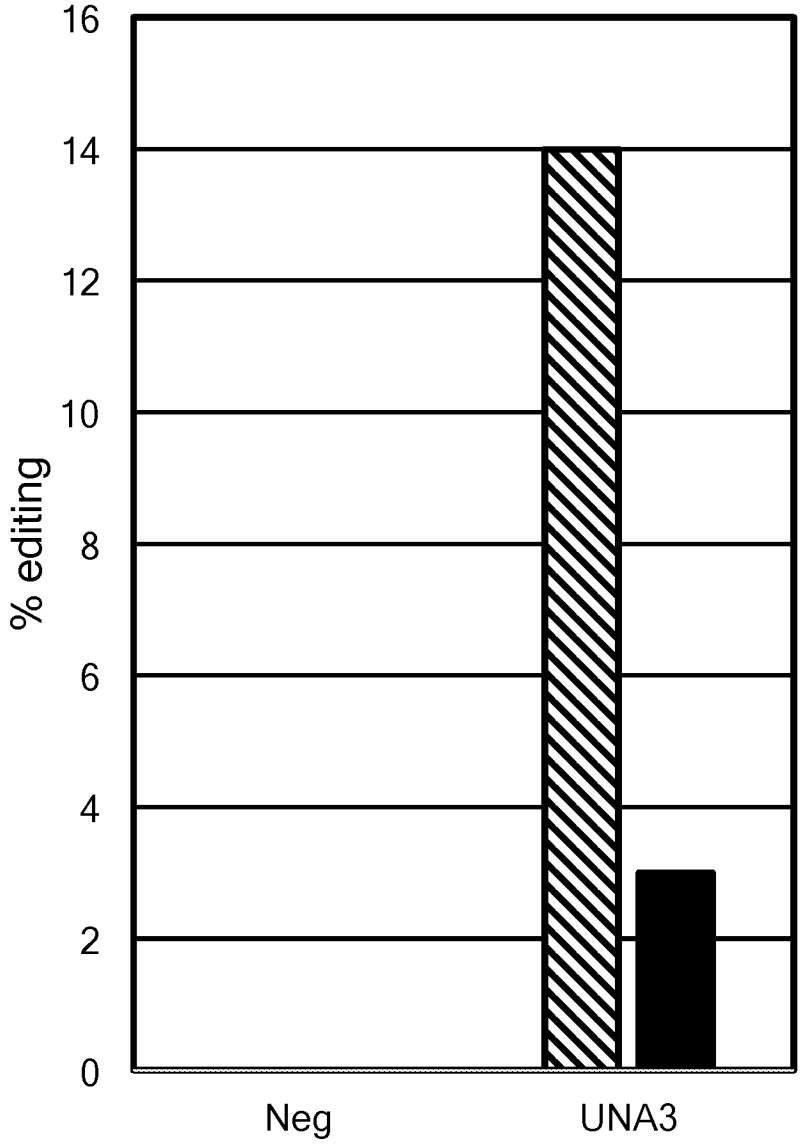


FIG. 11

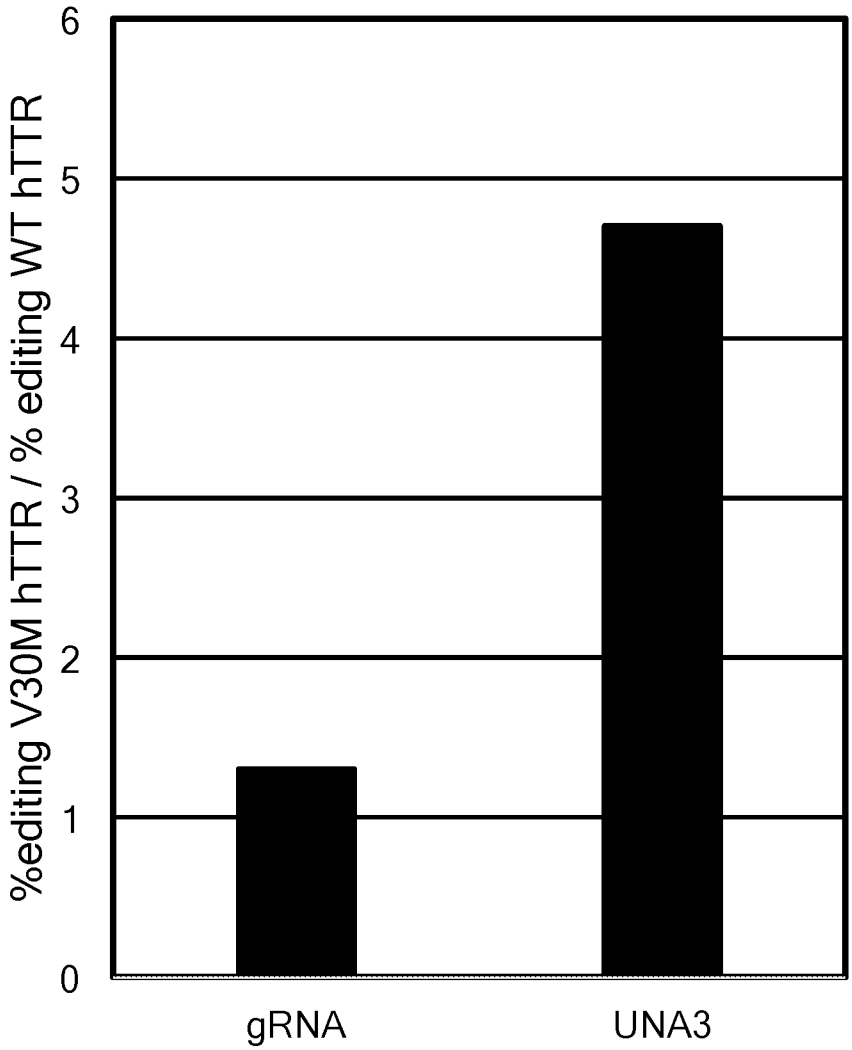


FIG. 12

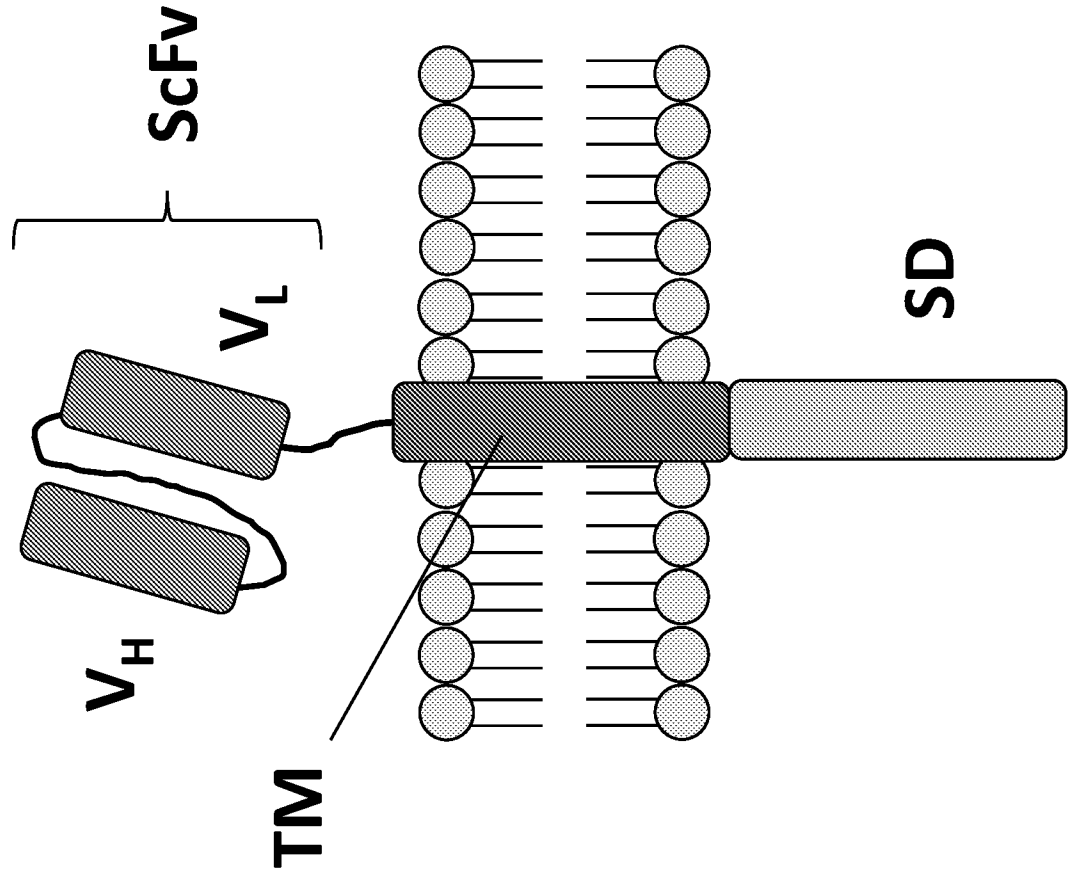


FIG. 13

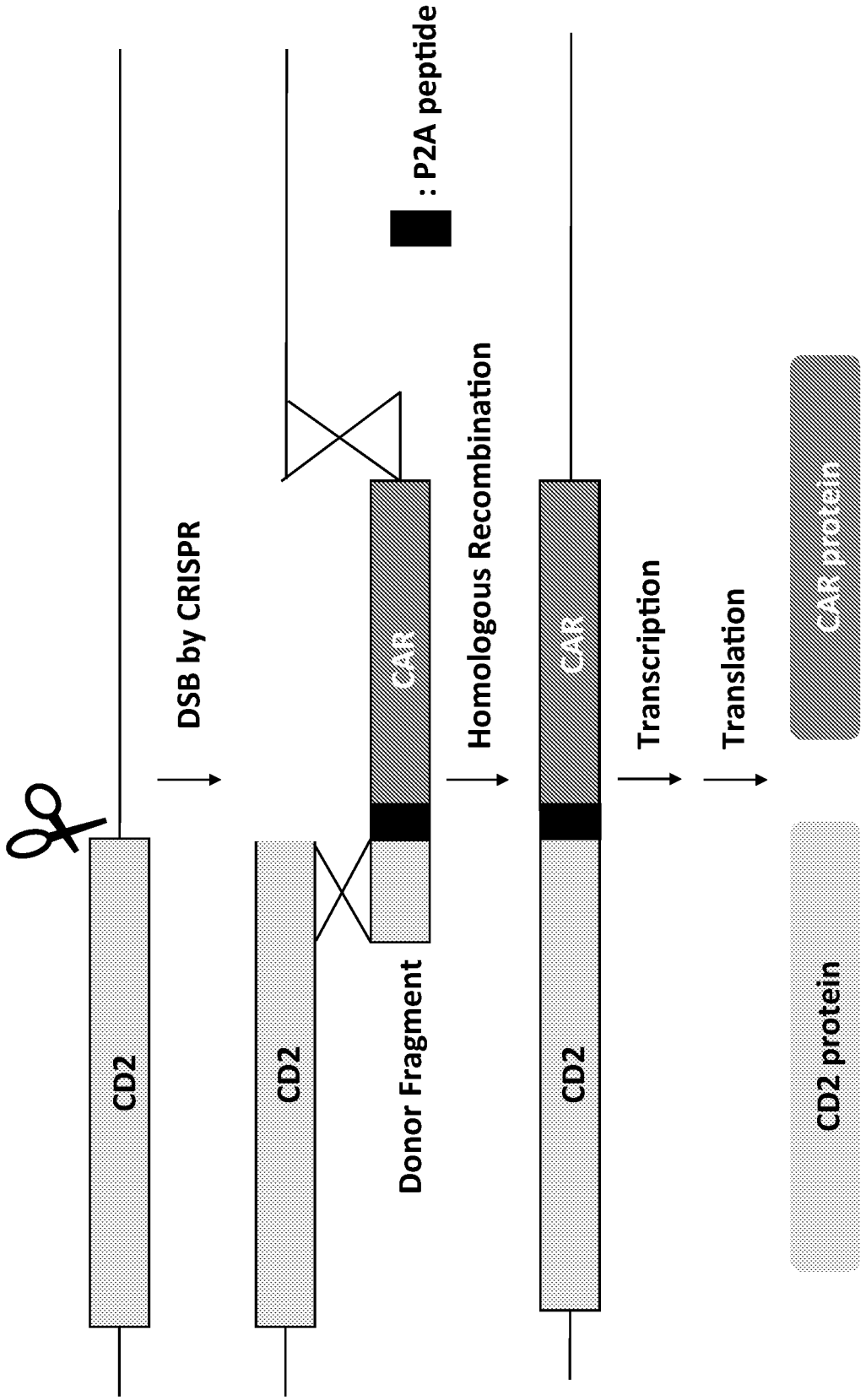

CD2
DSB by CRISPR
CD2
CAR
: P2A peptide
Donor Fragment
Homologous Recombination
CD2
CAR
Transcription
Translation
CD2 protein
CAR protein

FIG. 14

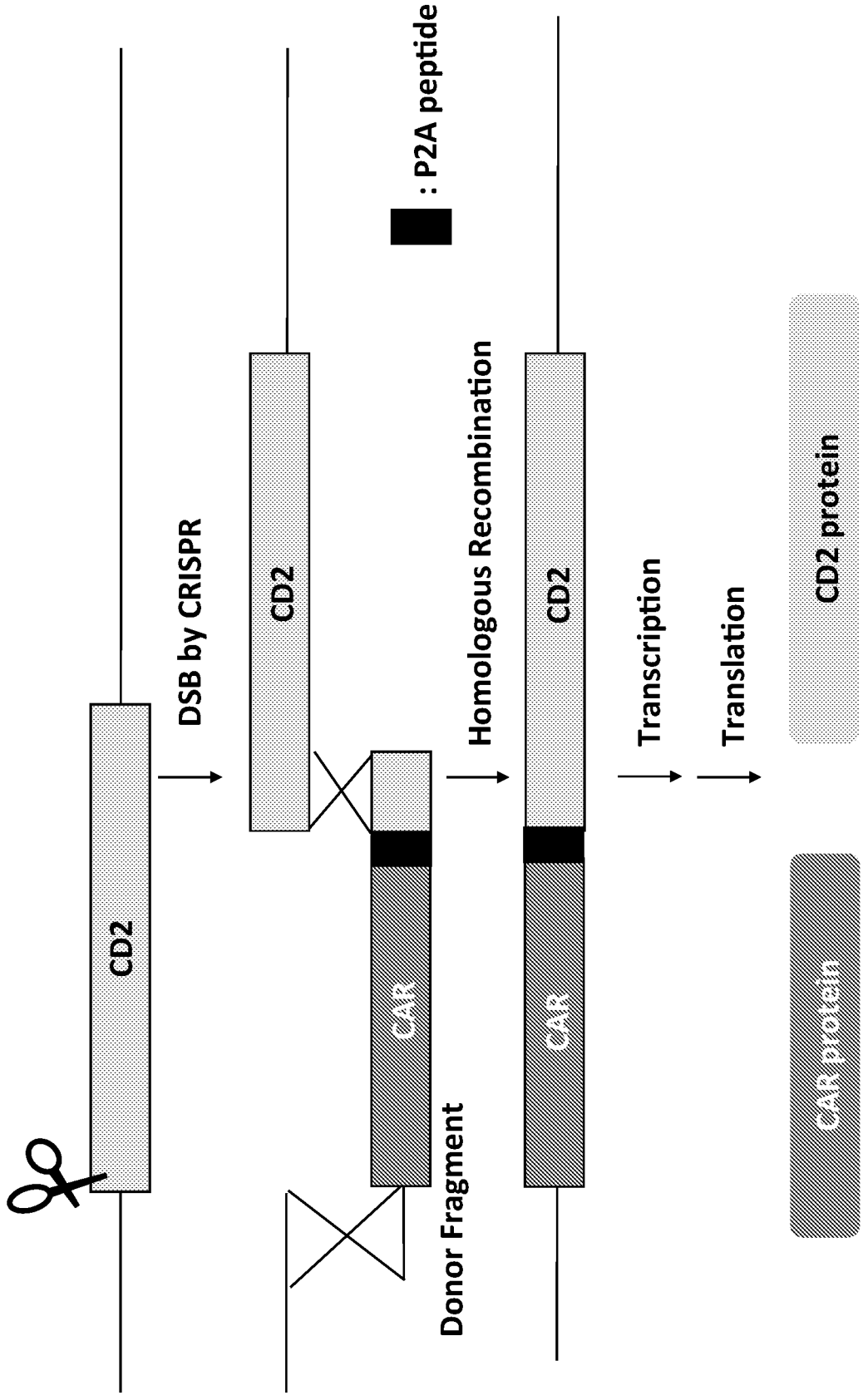

CD2
DSB by CRISPR
CD2
CAR
: P2A peptide
Donor Fragment
Homologous Recombination
CD2
CAR
Transcription
Translation
CD2 protein
CAR protein

FIG. 15