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(54) **NUCLEOBASE EDITORS COMPRISING GEOCAS9 AND USES THEREOF**

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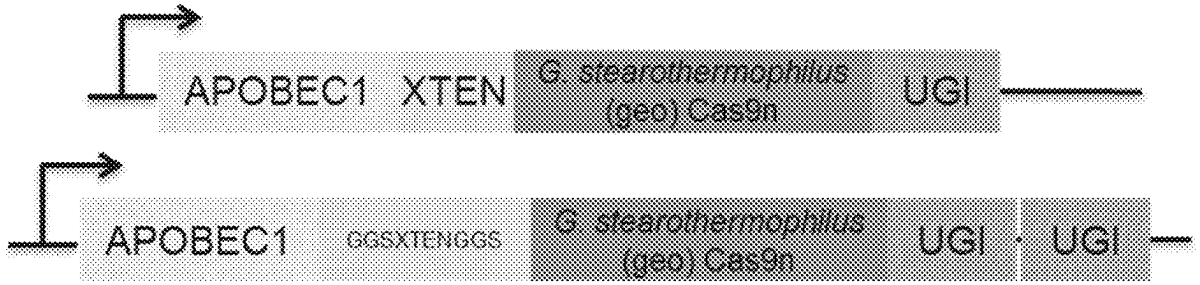
(57) **ABSTRACT**

Some aspects of this disclosure provide strategies, systems, reagents, methods, and kits that are useful for the targeted editing of nucleic acids or the modification of nucleic acids or proteins, including editing a single site within the genome of a cell or subject, e.g., within the human genome. In some embodiments, fusion proteins of nucleic acid programmable DNA binding proteins e.g., GeoCas9 or variants thereof, and effector domains, e.g., deaminase domains, are provided. In some embodiments, methods for targeted nucleic acid editing or protein modification are provided. In some embodiments, reagents and kits for the generation of targeted nucleic acid editing proteins, e.g., fusion proteins of a GeoCas9 and effector domains, are provided.

Specification includes a Sequence Listing.



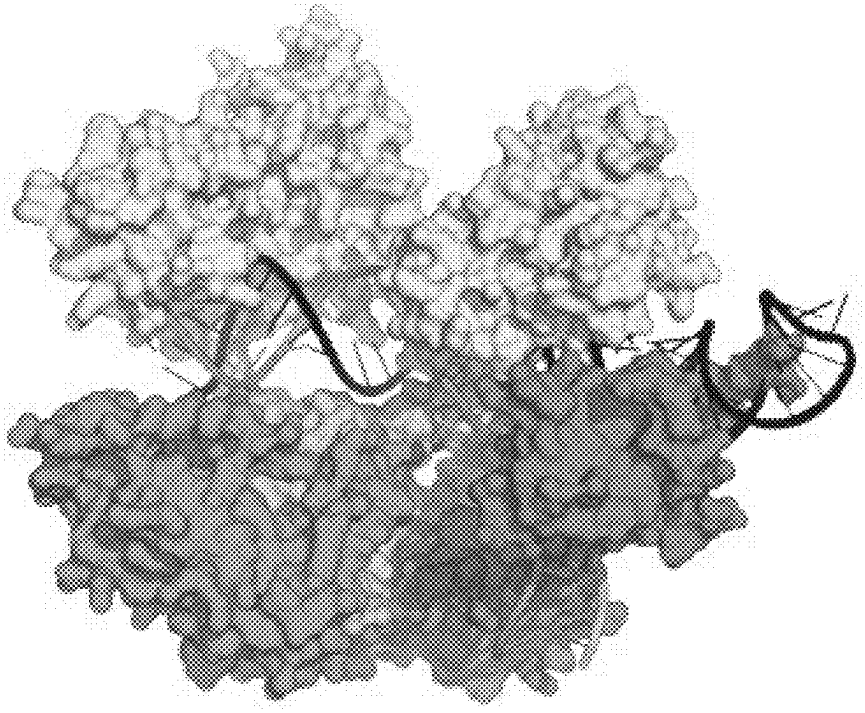


FIGURE 1

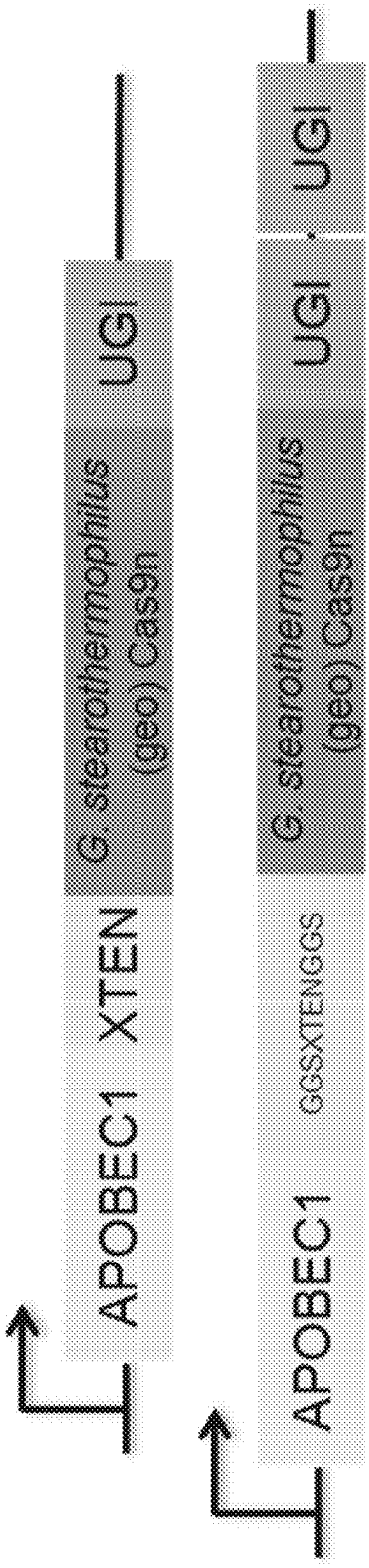

APOBEC1
XTEN
G. stearothermophilus
(geo) Cas9n
UGI
APOBEC1
GGSXTENGGS
G. stearothermophilus
(geo) Cas9n
UGI
UGI

FIGURE 2

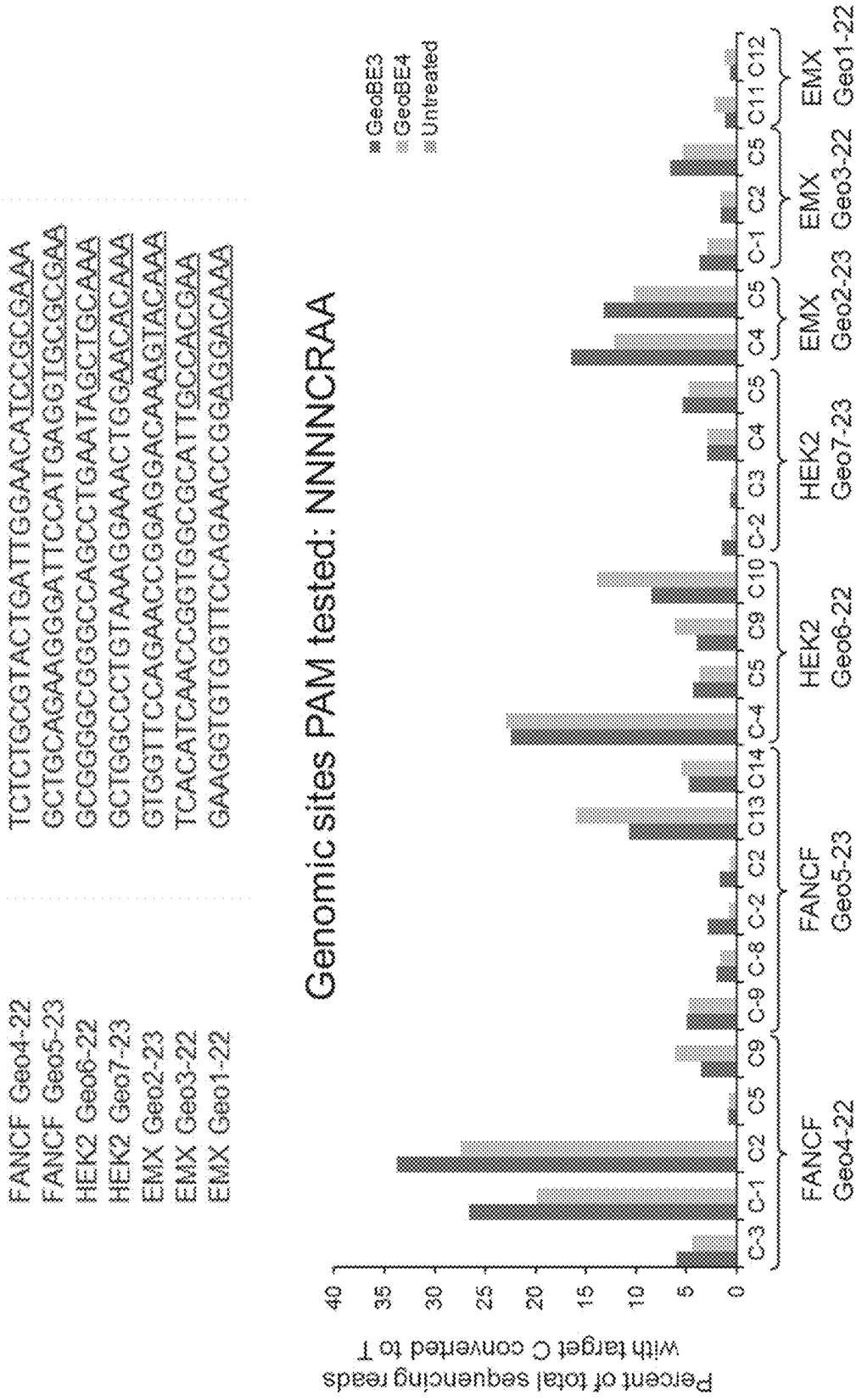

FANCF Geo4-22 TCTCTGCGTACTGATTGGAACATCCGCGAAA
FANCF Geo5-23 GCTGCAGAAGGGATTCCATGAGGTGCGCGAA
HEK2 Geo6-22 GCGGGGCGGGCCAGCCTGAATAGCTGCAAA
HEK2 Geo7-23 GCTGGCCCTGTAAAGGAAACTGGAACACAAA
EMX Geo2-23 GTGGTTCCAGAACCGGAGGACAAAGTACAAA
EMX Geo3-22 TCACATCAACCGGTGGCGCATTGCCACGAA
EMX Geo1-22 GAAGGTGTGGTTCCAGAACCGGAGGACAAA
Genomic sites PAM tested: NNNNCRAA
GeoBE3
GeoBE4
Untreated
Percent of total sequencing reads with target C converted to T
0
5
10
15
20
25
30
35
40
C-3 C-1 C2 C5 C9 C-9 C-8 C-2 C2 C13 C14 C-4 C5 C9 C10 C-2 C3 C4 C5 C4 C5 C-1 C2 C5 C11 C12
FANCF Geo4-22
FANCF Geo5-23
HEK2 Geo6-22
HEK2 Geo7-23
EMX Geo2-23
EMX Geo3-22
EMX Geo1-22

FIGURE 3

NUCLEOBASE EDITORS COMPRISING GEOCAS9 AND USES THEREOF

RELATED APPLICATIONS

[0001] This application is a division of and claims priority under 35 U.S.C. § 120 to U.S. patent application U.S. Ser. No. 17/289,665, filed Apr. 28, 2021, which is a national stage filing under 35 U.S.C. § 371 of International PCT Application, PCT/US2019/058678, filed Oct. 29, 2019, which claims priority under 35 U.S.C. § 119 (e) to U.S. Provisional Application, U.S. Ser. No. 62/752,225, filed on Oct. 29, 2018, each of which is incorporated herein by reference.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The contents of the electronic sequence listing (B119570047US02-SUBSEQ-TNG.xml; Size: 590,708 bytes; and Date of Creation: May 9, 2025) are herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Targeted editing of nucleic acid sequences, for example, the targeted cleavage or the targeted introduction of a specific modification into genomic DNA, is a highly promising approach for the study of gene function and also has the potential to provide new therapies for human genetic diseases. An ideal nucleic acid editing technology possesses three characteristics: (1) high efficiency of installing the desired modification; (2) minimal off-target activity; and (3) the ability to be programmed to edit precisely any site in a given nucleic acid, e.g., any site within the human genome. Current genome engineering tools, including engineered zinc finger nucleases (ZFNs), transcription activator like effector nucleases (TALENs), and most recently, the RNA-guided DNA endonuclease Cas9 (e.g., Cas9 from *S. pyogenes*), affect sequence-specific DNA cleavage in a genome.

[0004] CRISPR-Cas (CRISPR associated) systems are protein-RNA complexes that use an RNA molecule (sgRNA) as a guide to localize the complex to a target DNA sequence via base-pairing. In the natural systems, a Cas protein then acts as an endonuclease to cleave the targeted DNA sequence. The target DNA sequence must be both complementary to the sgRNA, and also contain a "protospacer-adjacent motif" (PAM) at the 3'-end of the complementary region in order for the system to function.

[0005] Among the known Cas proteins, *S. pyogenes* Cas9 (spCas9) has been mostly widely used as a tool for genome engineering. However there are disadvantages to using spCas9 for nucleic acid engineering. For example, spCas9 protein is a large, multi-domain protein, and it requires a specific protospacer-adjacent motif (e.g., 5'-NGG-3') sequence to bind and cleave a particular nucleic acid sequence.

[0006] This programmable cleavage can result in mutation of the DNA at the cleavage site via non-homologous end joining (NHEJ) or replacement of the DNA surrounding the cleavage site via homology-directed repair (HDR). Some drawbacks to the current technologies include the large size of engineered nucleases, such as Cas9, used for the modification of nucleic acids, the requirement of Cas9 proteins to bind adjacent to a specific protospacer-adjacent motif (PAM) sequence, and the stability of Cas9 proteins under different temperature conditions.

[0007] Accordingly, genome engineering tools with improved properties are desired.

SUMMARY OF THE INVENTION

[0008] Some aspects of the disclosure are based on the recognition that fusion proteins comprising Cas9 proteins with desired properties are useful for nucleic acid engineering applications. For example, advantages of using GeoCas9 as a recognition agent include (1) the sequence specificity of GeoCas9 can be easily altered by simply changing the sgRNA sequence; (2) GeoCas9 binds to its target sequence by denaturing the dsDNA, resulting in a stretch of DNA that is single-stranded and therefore a viable substrate for the deaminase; (3) GeoCas9 is a thermostable hololog of other Cas9 proteins; (4) GeoCas9 is smaller than many homologs of Cas9 proteins from other organisms; and (5) GeoCas9 recognizes and can bind to nucleic acid sequences comprising the PAM with differing specificity relative to a wild type GeoCas9.

[0009] Accordingly, some aspects of the disclosure provide fusion proteins comprising: (i) a GeoCas9; (ii) a cytidine deaminase domain; and (iii) a uracil glycosylase inhibitor (UGI) domain. As one example, the GeoCas9 may be a GeoCas9 nickase ("nGeoCas9" or "GeoCas9n"). The GeoCas9n may be configured to cut a nucleotide target strand of a nucleotide duplex, wherein the nucleotide target strand is the strand that binds to a gRNA of the GeoCas9 nickase. In some embodiments, nicking one of the DNA strands facilitates base editing efficiency. In other embodiments, the fusion proteins comprise a second UGI domain, which may further improve base editing efficiency. The disclosure further contemplates compositions comprising any of the fusion proteins provided herein and a guide RNA (gRNA) molecule, which may be used to target a specific nucleobase within a DNA molecule (e.g., genomic DNA molecule).

[0010] The summary above is meant to illustrate, in a non-limiting manner, some of the embodiments, advantages, features, and uses of the technology disclosed herein. Other embodiments, advantages, features, and uses of the technology disclosed herein will be apparent from the Detailed Description, the Drawings, the Examples, and the Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] FIG. **1** shows a homology model of a *Geobacillus stearothermophilus* Cas9 (GeoCas9) docked with DNA (Harrington et al.).

[0012] FIG. **2** shows schematic representatins of base editors containing GeoCas9. The top schematic shows a base editor 3 (BE3) with GeoCas9, referred to as GeoBE3, which comprises an N-terminal APOBEC1 cytidine deaminase linked to GeoCas9 via an XTEN linker, and a C-terminal UGI domain that is linked to the GeoCas9. The bottom schematic shows a base editor 4 (BE4) with GeoCas9, referred to as GeoBE4, which comprises an N-terminal APOBEC1 cytidine deaminase linked to GeoCas9 via a GGS-XTEN-GGS linker (SEQ ID NO: 436), and a two C-terminal UGI domains.

[0013] FIG. **3** shows base editing data for GeoBE3 and GeoBE4 on selected DNA sequences. Sequences FANCF

Geo4-22 (SEQ ID NO: 406); FANCF Geo5-23 (SEQ ID NO: 407); HEK2 Geo6-22 (SEQ ID NO: 408); HEK2 Geo7-23 (SEQ ID NO: 409); EMX Geo2-23 (SEQ ID NO: 410); EMX Geo2-22 (SEQ ID NO: 411); EMX Geo1-22 (SEQ ID NO: 412), shown from top to bottom, were edited using GeoBE3 and GeoBE4. The PAM sequence for each of the DNA target sequences is indicated by underlining. The PAM consensus sequence is 5'-NNNNCRAA-3' (SEQ ID NO: 79), where N is any nucleotide (e.g., A, T, C, or G), and R is A or G, as indicated by the IUPAC nucleotide code. The base editing results, indicating the percent of total sequencing reads where the indicated C is converted to T, is shown in the chart (bottom). For all target C positions, the PAM has been set as positions 21-28 (N21NNNCRAA28). The target sequences are numbered in relation to the PAM. For Example, the numbering convention for FANCF Geo4-22 is as follows: $T_{-3}C_{-2}T_{-1}C_1T_2G_3C_4G_5T_6A_7C_8T_9G_{10}A_{11}T_{12}T_{13}G_{14}G_{15}A_{16}A_{17}C_{18}A_{19}T_{20}C_{21}C_{22}G_{23}C_{24}G_{25}A_{26}A_{27}A_{28}$ (SEQ ID NO: 406). There are targeted Cs further downstream, which are labeled as negative positions. For each base before position 1, the numbering continues as −1, −2, −3, etc.

DEFINITIONS

[0014] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used in this invention: Singleton et al., *Dictionary of Microbiology and Molecular Biology* (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); *The Glossary of Genetics,* 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, *The Harper Collins Dictionary of Biology* (1991). As used herein, the following terms have the meanings ascribed to them unless specified otherwise.

a/an/the

[0015] As used herein and in the claims, the singular forms "a," "an," and "the" include the singular and the plural reference unless the context clearly indicates otherwise. Thus, for example, a reference to "an agent" includes a single agent and a plurality of such agents.

Base Editor (BE)

[0016] The term "base editor (BE)," or "nucleobase editor (NBE)," as used herein, refers to an agent comprising a polypeptide that is capable of making a modification to a base (e.g., A, T, C, G, or U) within a nucleic acid sequence (e.g., DNA or RNA). In some embodiments, the base editor is capable of deaminating a base within a nucleic acid. In some embodiments, the base editor is capable of deaminating a base within a DNA molecule. In some embodiments, the base editor is capable of deaminating a cytosine (C) in DNA. In some embodiments, the base editor is a fusion protein comprising a GeoCas9 fused to a cytidine deaminase domain. In some embodiments, the base editor comprises a GeoCas9 (e.g., catalytically inactive dGeoCas9 or GeoCas9 nickase). In some embodiments, the base editor comprises a GeoCas9 nickase (GeoCas9n) fused to a cytidine deaminase. In some embodiments, the base editor comprises a nuclease-inactive GeoCas9 (dGeoCas9) fused to a cytidine deaminase. In some embodiments, the base editor is fused to an inhibitor of base excision repair, for example, a UGI domain. In some embodiments, the base editor comprises a UGI protein fused to a cytidine deaminase. Where the base editor comprises a GeoCas9, the base editor may be referred to as a "GeoCas9 base editor" or simply as a "base editor." In addition, since the GeoCas9 base editors comprise fusion proteins, they may also be referred to as "GeoCas9 base editor fusion proteins."

Cas9

[0017] The term "Cas9" or "Cas9 nuclease" refers to an RNA-guided protein comprising a Cas9 domain, or a fragment thereof (e.g., a protein comprising an active, inactive, or partially active DNA cleavage domain of Cas9, and/or the gRNA binding domain of Cas9). A Cas9 nuclease is also referred to sometimes as a casn1 nuclease or a CRISPR (clustered regularly interspaced short palindromic repeat)-associated nuclease. CRISPR is an adaptive immune system that provides protection against mobile genetic elements (viruses, transposable elements and conjugative plasmids). CRISPR clusters contain spacers, sequences complementary to antecedent mobile elements, and target invading nucleic acids. CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In type II CRISPR systems correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the spacer. The target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3'-5' exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species. See, e.g., Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. *Science* 337:816-821 (2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. Cas9 nuclease sequences and structures are well known to those of skill in the art (see, e.g., "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti et al., J. J., McShan W. M., Ajdic D. J., Savic D. J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A. N., Kenton S., Lai H. S., Lin S. P., Qian Y., Jia H. G., Najar F. Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S. W., Roe B. A., Mclaughlin R. E., *Proc. Natl. Acad. Sci. U.S.A.* 98:4658-4663 (2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C. M., Gonzales K., Chao Y., Pirzada Z. A., Eckert M. R., Vogel J., Charpentier E., *Nature* 471:602-607 (2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. Science 337:816-821 (2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *Geobacillus stearothermophilus, S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and

sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference. In some embodiments, a Cas9 has an inactive (e.g., an inactivated) DNA cleavage domain, that is, the Cas9 is a nuclease inactive Cas9. In some embodiments, a Cas9 has a partially inactive DNA cleavage domain, that is, the Cas9 is a nickase that cleaves one strand of a DNA duplex.

[0018] An exemplary wild type Cas9 from *Streptococcus pyogenes* corresponds to the amino acid sequence of SEQ ID NO: 1 (Uniprot Reference Sequence: Q99ZW2).

(SEQ ID NO: 1)

```
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA
LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP
INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI
LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI
FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR
KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY
YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
NLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI
IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ
LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV
MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP
VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD
SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL
TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK
YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI
TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEV
QTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVE
KGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPK
YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
DNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDK
PIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGD
```

dCas9 or dGeoCas9

[0019] A "nuclease-inactivated Cas9 protein" may interchangeably be referred to as a "dCas9" protein (for nuclease "dead" Cas9). A "nuclease-inactivated GeoCas9" is also referred to herein as "dGeoCas9". Methods for generating a Cas9 protein (or a fragment thereof) having an inactive DNA cleavage domain are known (See, e.g., Jinek et al., *Science*. 337:816-821 (2012); Qi et al., "Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression" (2013) Cell. 28; 152 (5): 1173-83, the entire contents of each of which are incorporated herein by reference). For example, the DNA cleavage domain of Cas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of Cas9. For example, the mutations D8A and H582A, with respect to SEQ ID NO: 5 or SEQ ID NO: 6 inactivate the nuclease activity of GeoCas9. As an additional example the mutations D7A and H581A, with respect to SEQ ID NO: 3 inactivate the nuclease activity of GeoCas9. In some embodiments, the dCas9 protein is nuclease inactive GeoCas9 (dGeoCas9).

[0020] In some embodiments, dCas9 corresponds to, or comprises in part or in whole, a Cas9 amino acid sequence having one or more mutations that inactivate the Cas9 nuclease activity. For example, in some embodiments, a dCas9 comprises a D7A and/or a H581A mutation in the amino acid sequence provided in SEQ ID NO: 3, or at corresponding positions in any GeoCas9, for example, any one of the amino acid sequences provided in SEQ ID NOs: 5-8. For example, a dCas9 or Cas9 nickase comprises a D7A and/or a H581A mutation in the amino acid sequence provided in SEQ ID NO: 3. A skilled artisan would appreciate how to perform a sequene alignment of GeoCas9 sequences to identify corresponding amino acid sequences in GeoCas9 proteins.

[0021] In some embodiments, proteins comprising fragments of GeoCas9 are provided. For example, in some embodiments, a protein comprises one of two GeoCas9 proteins: (1) the gRNA binding domain of GeoCas9; or (2) the DNA cleavage domain of GeoCas9. In some embodiments, "GeoCas9 variants" are provided, which include mutant forms of GeoCas9, and/or fragments thereof. In some embodiments, a GeoCas9 variant shares homology to GeoCas9, or a fragment thereof. For example, in some embodiments, a GeoCas9 variant is at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to a wild type GeoCas9, such as any one of the GeoCas9 amino acid sequences set forth in any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 variant has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acid changes compared to a wild-type GeoCas9, such as any one of the GeoCas9 amino acid sequences set forth in any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 variant comprises a fragment of a GeoCas9 protein (e.g., a gRNA binding domain and/or a DNA-cleavage domain), such that the fragment is at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to the corresponding

fragment of wild-type GeoCas9. In some embodiments, the fragment is is at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% identical, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid length of a corresponding wild-type GeoCas9, and is capable of associating with (e.g., forming a complex with) a nucleic acid (e.g., RNA), such as a guide nucleic acid (gRNA). In some embodiments, the GeoCas9 fragment is at least 100 amino acids in length. In some embodiments, the fragment is at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1060, at least 1070, or at least 1080 amino acids in length, and is capable of associating with (e.g., forming a complex with) a nucleic acid (e.g., RNA), such as a guide nucleic acid (gRNA). In some embodiments, Cas9 corresponds to the GeoCas9 of any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, Cas9 corresponds to GeoCas9 of SEQ ID NO: 2. In some embodiments, wild-type Cas9 corresponds to GeoCas9 of SEQ ID NO: 3.

[0022] In some embodiments, GeoCas9 comprises a D7A mutation, while the residue at position 581 remains a histidine in the amino acid sequence provided in SEQ ID NO: 3 or at corresponding positions in any GeoCas9, for example, any one of the amino acid sequences provided in SEQ ID NOs: 5-8. Without wishing to be bound by any particular theory, the presence of the catalytic residue H581, allows GeoCas9 to cleave the non-edited (e.g., non-deaminated) strand containing a G opposite the targeted C. Restoration of H581 (e.g., from A581) in a GeoCas9 D7A and H581A mutant does not result in the cleavage of the target strand containing the C. Such GeoCas9 variants are able to generate a single-strand DNA break (nick) at a specific location based on the gRNA-defined target sequence, leading to repair of the non-edited strand, ultimately resulting in a G to A change on the non-edited strand. Briefly, the C of a C-G basepair can be deaminated to a U by a deaminase, e.g., an APOBEC deamonase. Nicking the non-edited strand, having the G, facilitates removal of the G via mismatch repair mechanisms. UGI inhibits UDG, which prevents removal of the U.

[0023] In other embodiments, dGeoCas9 or GeoCas9 nickase variants having mutations other than D7A and H581A, corresponding to SEQ ID NO: 3 are provided, which, e.g., result in nuclease inactivated GeoCas9 (dGeoCas9) or GeoCas9 nickase. Such mutations, by way of example, include other amino acid substitutions at D7 and/or H581, in the amino acid sequence provided in SEQ ID NO: 3 or at corresponding positions in any GeoCas9, for example, any one of the amino acid sequences provided in SEQ ID NOs: 5-8. Additional examples include other mutations within the nuclease domains of GeoCas9 (e.g., substitutions in the HNH nuclease subdomain and/or the RuvC1 subdomain).

[0024] In some embodiments, variants or homologues of GeoCas9 (e.g., variants of any one of SEQ ID NOs: 2, 3, or 5-8) are provided which are at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, variants of GeoCas9 (e.g., variants of any one of SEQ ID NOs: 2, 3, or 5-8) are provided having amino acid sequences which are shorter, or longer than any one of SEQ ID NOs: 2, 3, or 5-8, by at least 5 amino acids, by at least 10 amino acids, by at least 15 amino acids, by at least 20 amino acids, by at least 25 amino acids, by at least 30 amino acids, by at least 40 amino acids, by at least 50 amino acids, by at least 75 amino acids, by at least 100 amino acids or more.

[0025] In some embodiments, GeoCas9 fusion proteins as provided herein comprise the full-length amino acid sequence of a GeoCas9 protein, e.g., any one of the GeoCas9 sequences provided herein. In other embodiments, however, fusion proteins as provided herein do not comprise a full-length GeoCas9 sequence, but only a fragment thereof. For example, in some embodiments, a GeoCas9 fusion protein provided herein comprises a GeoCas9 fragment, wherein the fragment is capable of binding a crRNA and tracrRNA or sgRNA, but does not comprise a functional nuclease domain, e.g., in that it comprises only a truncated version of a nuclease domain or no nuclease domain at all. Exemplary amino acid sequences of suitable GeoCas9 proteins and GeoCas9 fragments are provided herein, and additional suitable sequences of GeoCas9 proteins and fragments thereof will be apparent to those of skill in the art.

CRISPR

[0026] CRISPR is a family of DNA sequences (i.e., CRISPR clusters) in bacteria and archaea that represent snippets of prior infections by a virus that have invaded the prokaryote. The snippets of DNA are used by the prokaryotic cell to detect and destroy DNA from subsequent attacks by similar viruses and effectively compose, along with an array of CRISPR-associated proteins (including Cas9 and homologs thereof) and CRISPR-associated RNA, a prokaryotic immune defense system. In nature, CRISPR clusters are transcribed and processed into CRISPR RNA (crRNA). In certain types of CRISPR systems (e.g., type II CRISPR systems), correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc) and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA.

[0027] Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular dsDNA target complementary to the RNA. Specifically, the target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3'-5' exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gNRA") can be engineered so as to incorporate aspects of both the crRNA and tracrRNA into a single RNA species—the guide RNA. See, e.g., Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. Science 337:816-821 (2012), the entire contents of which is hereby incorporated by reference. Cas9 recognizes a short motif in the CRISPR repeat sequences (the PAM or protospacer adjacent motif) to help distinguish self versus non-self. CRISPR biology, as well as Cas9 nuclease sequences and structures are well known to those of skill in the art (see, e.g., "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti et al., J. J., McShan W. M., Ajdic D. J., Savic D. J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A. N., Kenton S., Lai H. S., Lin S. P., Qian Y., Jia H. G., Najar F. Z., Ren Q., Zhu H., Song L., White J., Yuan

X., Clifton S. W., Roe B. A., McLaughlin R. E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663 (2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C. M., Gonzales K., Chao Y., Pirzada Z. A., Eckert M. R., Vogel J., Charpentier E., Nature 471:602-607 (2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. Science 337:816-821 (2012), the entire contents of each of which are incorporated herein by reference). Cas9 orthologs have been described in various species, including, but not limited to, *S. pyogenes* and *S. thermophilus*. Additional suitable Cas9 nucleases and sequences will be apparent to those of skill in the art based on this disclosure, and such Cas9 nucleases and sequences include Cas9 sequences from the organisms and loci disclosed in Chylinski, Rhun, and Charpentier, "The tracrRNA and Cas9 families of type II CRISPR-Cas immunity systems" (2013) RNA Biology 10:5, 726-737; the entire contents of which are incorporated herein by reference.

[0028] In certain types of CRISPR systems (e.g., type II CRISPR systems), correct processing of pre-crRNA requires a trans-encoded small RNA (tracrRNA), endogenous ribonuclease 3 (rnc), and a Cas9 protein. The tracrRNA serves as a guide for ribonuclease 3-aided processing of pre-crRNA. Subsequently, Cas9/crRNA/tracrRNA endonucleolytically cleaves linear or circular nucleic acid target complementary to the RNA. Specifically, the target strand not complementary to crRNA is first cut endonucleolytically, then trimmed 3'-5' exonucleolytically. In nature, DNA-binding and cleavage typically requires protein and both RNAs. However, single guide RNAs ("sgRNA", or simply "gRNA") can be engineered so as to incorporate embodiments of both the crRNA and tracrRNA into a single RNA species—the guide RNA.

[0029] In general, a "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus. The tracrRNA of the system is complementary (fully or partially) to the tracr mate sequence present on the guide RNA.

Deaminase

[0030] The term "deaminase" or "deaminase domain," as used herein, refers to a protein or enzyme that catalyzes a deamination reaction. In some embodiments, the deaminase or deaminase domain is a naturally-occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the deaminase or deaminase domain is a variant of a naturally-occurring deaminase from an organism, that does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring deaminase from an organism.

[0031] In some embodiments, the deaminase or deaminase domain is a cytidine deaminase, catalyzing the hydrolytic deamination of cytidine or deoxycytidine to uridine or deoxyuridine, respectively. In some embodiments, the deaminase or deaminase domain is a cytidine deaminase domain, catalyzing the hydrolytic deamination of cytosine to uracil. In some embodiments, the cytidine deaminase catalyzes the hydrolytic deamination of cytidine or cytosine in deoxyribonucleic acid (DNA). In some embodiments, the cytidine deaminase domain comprises the amino acid sequence of any one disclosed herein. In some embodiments, the cytidine deaminase or cytidine deaminase domain is a naturally-occurring cytidine deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse. In some embodiments, the cytidine deaminase or cytidine deaminase domain is a variant of a naturally-occurring cytidine deaminase from an organism that does not occur in nature. For example, in some embodiments, the cytidine deaminase or cytidine deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring cytidine deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse.

[0032] In some embodiments, the deaminase or deaminase domain is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase, catalyzing the hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (e.g., engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium. In some embodiments, the deaminase or deaminase domain is a variant of a naturally-occurring deaminase from an organism. In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring deaminase. In some embodiments, the adenosine deaminase is from a bacterium, such as *E. coli, S. aureus, S. typhi, S. putrefaciens, H. influenzae*, or *C. crescentus.*

Effective Amount

[0033] The term "effective amount," as used herein, refers to an amount of a biologically active agent that is sufficient to elicit a desired biological response. For example, in some embodiments, an effective amount of a nuclease may refer to the amount of the nuclease that is sufficient to induce cleavage of a target site specifically bound and cleaved by the nuclease. In some embodiments, an effective amount of a fusion protein provided herein, e.g., of a fusion protein comprising a GeoCas9 and a nucleic acid editing domain

(e.g., a deaminase domain), may refer to the amount of the fusion protein that is sufficient to induce editing of a target site specifically bound and edited by the fusion protein. As will be appreciated by the skilled artisan, the effective amount of an agent, e.g., a fusion protein, a nuclease, a deaminase, a recombinase, a hybrid protein, a protein dimer, a complex of a protein (or protein dimer) and a polynucleotide, or a polynucleotide, may vary depending on various factors as, for example, on the desired biological response, e.g., on the specific allele, genome, or target site to be edited, on the cell or tissue being targeted, and on the agent being used.

Effector Domain

[0034] The term, as used herein, "effector domain," or the equivalent terms "nucleobase modification moiety" or "nucleic acid effector domain," embraces any protein, enzyme, or polypeptide (or functional fragment thereof) which is capable of modifying a DNA or RNA molecule. Nucleobase modification moieties can be naturally occurring, or can be recombinant. For example, a nucleobase modification moiety can include one or more DNA repair enzymes, for example, and an enzyme or protein involved in base excision repair (BER), nucleotide excision repair (NER), homology-dependent recombinational repair (HR), non-homologous end-joining repair (NHEJ), microhomology end-joining repair (MMEJ), mismatch repair (MMR), direct reversal repair, or other known DNA repair pathway. A nucleobase modification moiety can have one or more types of enzymatic activities, including, but not limited to endonuclease activity, polymerase activity, ligase activity, replication activity, proofreading activity, or a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain.

Functional Equivalent

[0035] The term "functional equivalent" refers to a second biomolecule that is equivalent in function, but not necessarily equivalent in structure to a first biomolecule. For example, a "GeoCas9 equivalent" refers to a protein that has the same or substantially the same functions as GeoCas9, but not necessarily the same amino acid sequence. In the context of the disclosure, the specification refers throughout to "a protein X, or a functional equivalent thereof." In this context, a "functional equivalent" of protein X embraces any homolog, paralog, fragment, naturally occurring, engineered, mutated, or synthetic version of protein X which bears an equivalent function.

Fusion Protein

[0036] The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins (e.g., a GeoCas9 fusion protein, which comprises a GeoCas9 domain fused to a nucleic acid effector domain). One protein may be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an "amino-terminal fusion protein" or a "carboxy-terminal fusion protein," respectively. A protein may comprise different domains, for example, a nucleic acid binding domain (e.g., the gRNA binding domain of GeoCas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain or a catalytic domain of a nucleic-acid editing protein. Another example includes a GeoCas9 or equivalent thereof fused to a deaminase enzyme. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

GeoCas9

[0037] The term "GeoCas9" refers to an RNA-guided protein from a bacterium of the genus *Geobacillus* (e.g., *G. stearothermophilus*), including variants and/or fragments thereof, that are capable of associating with a nucleic acid (e.g., RNA), such as a guide nucleic acid (gRNA). In some embodiments, the GeoCas9 comprises a variant of the amino acid sequence of any one of SEQ ID NOs: 2, 3, or 5-8 that is capable of associating with a nucleic acid (e.g., RNA), such as a guide nucleic acid (gRNA). In some embodiments, the GeoCas9 comprises a fragment of the amino acid sequence of any one of SEQ ID NOs: 2, 3, or 5-8 that is capable of associating with a nucleic acid (e.g., RNA), such as a guide nucleic acid (gRNA). In some embodiments, the GeoCas9 comprises the amino acid sequence of any one of SEQ ID NOS: 2, 3, or 5-8.

[0038] In some embodiments, the GeoCas9 is from the genus *Geobacillus* (e.g., *G. stearothermophilus, G. thermodenitrificans, G. caldoxylosilyticus, G. galactosidasius, G. icigianus, G. jurassicus, G. kaustophilus, G. lituanicus, G. subterraneus, G. thermantarcticus, G. thermocatenulatus, G. thermoglucosidasius, G. thermoleovorans, G. toebii, G. uzenensis*, or *G. vulcani*), or variant thereof that binds to a guide RNA in order to bind a target nucleic acid (e.g., DNA) sequence. In some embodiments, the GeoCas9 is from *Geobacillus stearothermophilus*. GeoCas9 from *Geobacillus stearothermophilus* catalyzes RNA-guided DNA cleavage at elevated temperatures (e.g., up to 70° C.), and this thermostablility and apparent resistance to degradation in vivo may provide expanded utility when GeoCas9 is incorporated into the CRISPR-Cas9 system (see, e.g., "A thermostable Cas9 with increased lifetime in human plasma" Harrington L. B., Paez-Espino D., Staahl B. T., Chen J. C., Ma E., Kyrpides N. C., Doudna J. A. Nature Communications 8, 1424 (2017); "Characterizing a thermostable Cas9 for bacterial genome editing and silencing" Mougiakos I., Mohanraju P., Bosma E. F., Vrouwe V., Bou M. F., Naduthodi M. I. S., Gussak A., Brinkman R. B. L., van Kranenburg R., van der Oost J. Nature Communications 8, 1647 (2017); and "Type II-C CRIPSR-Cas9 Biology, Mechanism, and Application" Mir A., Edraki A., Lee J., Sontheimer E. J. ACS Chemical Biology doi: 10.1021/acschembio.7b00855 (2017), the entire contents of each of which are incorporated herein by reference).

Gene of Interest

[0039] The term "gene of interest," as used herein, refers to a nucleic acid construct comprising a nucleotide sequence

encoding a gene product of interest, for example, a gene product (e.g., a genome editor or component/domain thereof) to be evolved in a continuous evolution process as provided herein.

Gene Function

[0040] The term "function of a gene of interest," as interchangeably used with the term "gene function" or "activity of a gene of interest," refers to a function or activity of a gene product, for example, a nucleic acid, or a protein, encoded by the gene of interest. For example, a function of a gene of interest may be an enzymatic activity (e.g., an enzymatic activity resulting in the generation of a reaction product, phosphorylation activity, phosphatase activity, etc.), an ability to activate transcription (e.g., transcriptional activation activity targeted to a specific promoter sequence), a bond-forming activity, (e.g., an enzymatic activity resulting in the formation of a covalent bond), or a binding activity (e.g., a protein, DNA, or RNA binding activity).

Linker

[0041] The term "linker," as used herein, refers to a chemical group or a molecule linking two molecules or moieties, e.g., two domains of a fusion protein, such as, for example, a GeoCas9 and a nucleic acid editing domain (e.g., a deaminase domain). A linker may be, for example, an amino acid sequence, a peptide, or a polymer of any length and composition. In some embodiments, a linker joins an RNA-guided protein, including a GeoCas9, and a deaminase domain (e.g., a cytidine deaminase domain). In some embodiments, a linker joins a GeoCas9 and a UGI domain. Typically, the linker is positioned between, or flanked by, two groups, molecules, or other moieties and connected to each one via a covalent bond, thus connecting the two. In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein). In some embodiments, the linker is an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is 1-100 amino acids in length, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 30-35, 35-40, 40-45, 45-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or 150-200 amino acids in length. Longer or shorter linkers are also contemplated.

Mutation

[0042] The term "mutation," as used herein, refers to a substitution of a residue within a sequence, e.g., a nucleic acid or amino acid sequence, with another residue, or a deletion or insertion of one or more residues within a sequence. Mutations are typically described herein by identifying the original residue followed by the position of the residue within the sequence and by the identity of the newly substituted residue. Various methods for making the amino acid substitutions (mutations) provided herein are well known in the art, and are provided by, for example, Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)). Mutations can include a variety of categories, such as single base polymorphisms, microduplication regions, indel, and inversions, and is not meant to be limiting in any way. Mutations can include "loss-of-function" mutations which is the normal result of a mutation that reduces or abolishes a protein activity. Most loss-of-function mutations are recessive, because in a heterozygote the second chromosome copy carries an unmutated version of the gene coding for a fully functional protein whose presence compensates for the effect of the mutation. There are some exceptions where a loss-of-function mutation is dominant, one example being haploinsufficiency, where the organism is unable to tolerate the approximately 50% reduction in protein activity suffered by the heterozygote. This is the explanation for a few genetic diseases in humans. Mutations also embrace "gain-of-function" mutations, which is one which confers an abnormal activity on a protein or cell that is otherwise not present in a normal condition. Many gain-of-function mutations are in regulatory sequences rather than in coding regions, and can therefore have a number of consequences. For example, a mutation might lead to one or more genes being expressed in the wrong tissues, these tissues gaining functions that they normally lack. Alternatively the mutation could lead to overexpression of one or more genes involved in control of the cell cycle, thus leading to uncontrolled cell division and hence to cancer. Because of their nature, gain-of-function mutations are usually dominant.

napDNAbp

[0043] As used herein, the term "nucleic acid programmable DNA binding protein" or "napDNAbp," of which Cas9 and GeoCas9 are examples, refers to a protein which use RNA:DNA hybridization to target and bind to specific sequences in a DNA molecule. Each napDNAbp is associated with at least one guide nucleic acid (e.g., guide RNA), which localizes the napDNAbp to a DNA sequence that comprises a DNA strand (i.e., a target strand) that is complementary to the guide nucleic acid, or a portion thereof (e.g., the protospacer of a guide RNA). In other words, the guide nucleic-acid "programs" the napDNAbp (e.g., Cas9, GeoCas9, or equivalent) to localize and bind to a complementary sequence.

[0044] Without being bound by theory, the binding mechanism of a napDNAbp-guide RNA complex, in general, includes the step of forming an R-loop whereby the napDNAbp induces the unwinding of a double-strand DNA target, thereby separating the strands in the region bound by the napDNAbp. The guideRNA protospacer then hybridizes to the "target strand." This displaces a "non-target strand" that is complementary to the target strand, which forms the single strand region of the R-loop. In some embodiments, the napDNAbp includes one or more nuclease activities, which then cut the DNA leaving various types of lesions. For example, the napDNAbp may comprises a nuclease activity that cuts the non-target strand at a first location, and/or cuts the target strand at a second location. Depending on the nuclease activity, the target DNA can be cut to form a "double-stranded break" whereby both strands are cut. In other embodiments, the target DNA can be cut at only a single site, i.e., the DNA is "nicked" on one strand. Exemplary napDNAbp with different nuclease activities include "Cas9 nickase" ("nCas9") and a deactivated Cas9 having no nuclease activities ("dead Cas9" or "dCas9"). Exemplary sequences for these and other napDNAbp are provided herein.

[0045] The related term "RNA-programmable nuclease," and "RNA-guided nuclease" are used interchangeably herein and refer to a nuclease that forms a complex with (e.g., binds or associates with) one or more RNA that is not a target for cleavage (e.g., a Cas9 or homolog or variant

thereof). In some embodiments, an RNA-programmable nuclease, when in a complex with an RNA, may be referred to as a nuclease: RNA complex. Typically, the bound RNA (s) is referred to as a guide RNA (gRNA). gRNAs can exist as a complex of two or more RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), though "gRNA" is used interchangeabley to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (e.g., and directs binding of a Cas9 (or equivalent) complex to the target); and (2) a domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA, and comprises a stem-loop structure. For example, in some embodiments, domain (2) is homologous to a tracrRNA as depicted in FIG. 1E of Jinek et al., *Science* 337:816-821 (2012), the entire contents of which is incorporated herein by reference. Other examples of gRNAs (e.g., those including domain 2) can be found in U.S. Provisional Patent Application, U.S. Ser. No. 61/874,682, filed Sep. 6, 2013, entitled "Switchable Cas9 Nucleases And Uses Thereof," and U.S. Provisional Patent Application, U.S. Ser. No. 61/874,746, filed Sep. 6, 2013, entitled "Delivery System For Functional Nucleases," the entire contents of each are hereby incorporated by reference in their entirety. In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an "extended gRNA." For example, an extended gRNA will, e.g., bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease: RNA complex. In some embodiments, the RNA-programmable nuclease is the (CRISPR-associated system) Cas9 endonuclease, for example Cas9 (Csn1) from *Streptococcus pyogenes* (see, e.g., "Complete genome sequence of an M1 strain of *Streptococcus pyogenes*." Ferretti J. J., McShan W. M., Ajdic D. J., Savic D. J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A. N., Kenton S., Lai H. S., Lin S. P., Qian Y., Jia H. G., Najar F. Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S. W., Roe B. A., Mclaughlin R. E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663 (2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C. M., Gonzales K., Chao Y., Pirzada Z. A., Eckert M. R., Vogel J., Charpentier E., Nature 471:602-607 (2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J. A., Charpentier E. Science 337:816-821 (2012), the entire contents of each of which are incorporated herein by reference.

[0046] Because RNA-programmable nucleases (e.g., Cas9 or GeoCas9) use RNA: DNA hybridization to target DNA cleavage sites, these proteins are able to be targeted, in principle, to any sequence specified by the guide RNA. Methods of using RNA-programmable nucleases, such as Cas9, for site-specific cleavage (e.g., to modify a genome) are known in the art (see e.g., Cong, L. et al. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819-823 (2013); Mali, P. et al. RNA-guided human genome engineering via Cas9. Science 339, 823-826 (2013); Hwang, W. Y. et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nature biotechnology 31, 227-229 (2013); Jinek, M. et al. RNA-programmed genome editing in human cells. *eLife* 2, e00471 (2013); Dicarlo, J. E. et al. Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic acids research* (2013); Jiang, W. et al. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature biotechnology* 31, 233-239 (2013); the entire contents of each of which are incorporated herein by reference).

Non-Naturally Occurring

[0047] The terms "non-naturally occurring" or "engineered" are used interchangeably and indicate the involvement of the hand of man. The terms, when referring to nucleic acid molecules or polypeptides (e.g., GeoCas9 or single-stranded DNA binding protein) mean that the nucleic acid molecule or the polypeptide is at least substantially free from at least one other component with which they are naturally associated in nature and/or as found in nature (e.g., an amino acid sequence not found in nature).

Nickase

[0048] The term "nickase" refers to a napDNAbp (e.g., GeoCas9) with one of the two nuclease domains inactivated. This enzyme is capable of cleaving only one strand of a target DNA.

nCas9 or nGeoCas9

[0049] The term "Cas9 nickase," as used herein, refers to a Cas9 protein that is capable of cleaving only one strand of a duplexed nucleic acid molecule (e.g., a duplexed DNA molecule). In some embodiments, the Cas9 nickase is a GeoCas9 nickase. In some embodiments, a GeoCas9 nickase comprises a D7A mutation and has a histidine at position H581 of SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as any one of SEQ ID NOs: 5-8. For example, a GeoCas9 nickase may comprise the amino acid sequence as set forth in SEQ ID NO: 8. Such a GeoCas9 nickase (GeoCas9n) has an active HNH nuclease domain and is able to cleave the non-targeted strand of DNA, i.e., the strand bound by the gRNA in a GeoCas9n: gRNA complex. Further, such a GeoCas9 nickase has an inactive RuvC nuclease domain and is not able to cleave the targeted strand of the DNA, i.e., the strand where base editing is desired. In some embodiments, the Cas9 nickase comprises the amino acid sequence of SEQ ID NO: 2 or a variant thereof, for example any variant thereof provided herein.

GeoCas9n (H8A):

(SEQ ID NO: 8)

MKYKIGLAIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPR

RLARSARRRLRRRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQL

RVEALDRKLNNDELARILLHLAKRRGFRSNRKSERTNKENSTMLKHIEEN

QSILSSYRTVAEMVVKDPKFSLHKRNKEDNYTNTVARDDLEREIKLIFAK

QREYGNIVCTEAFEHEYISIWASQRPFASKDDIEKKVGFCTFEPKEKRAP

KATYTFQSFTVWEHINKLRLVSPGGIRALTDDERRLIYKQAFHKNKITFH

DVRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFLELGAYHKIRKAIDSVY

GKGAAKSFRPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLADK

-continued

VYDEELIEELLNLSFSKFGHLSLKALRNILPYMEQGEVYSTACERAGYTF

TGPKKKQKTVLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIE

LARELSQSFDERRKMQKEQEGNRKKNETAIRQLVEYGLTLNPTGLDIVKF

KLWSEQNGKCAYSLQPIEIERLLEPGYTEVD**H**VIPYSRSLDDSYTNKVLV

LTKENREKGNRTPAEYLGLGSERWQQFETFVLTNKQFSKKKRDRLLRLHY

DENEENEFKNRNLNDTRYISRFLANFIREHLKFADSDDKQKVYTVNGRIT

AHLRSRWNFNKNREESNLHHAVDAAIVACTTPSDIARVTAFYQRREQNKE

LSKKTDPQFPQPWPHFADELQARLSKNPKESIKALNLGNYDNEKLESLQP

VFVSRMPKRSITGAAHQETLRRYIGIDERSGKIQTVVKKKLSEIQLDKTG

HFPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGELGPIIR

TIKIIDTTNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPIYTIDMMK

GILPNKAIEPNKPYSEWKEMTEDYTFRFSLYPNDLIRIEFPREKTIKTAV

GEEIKIKDLFAYYQTIDSSNGGLSLVSHDNNFSLRSIGSRTLKRFEKYQV

DVLGNIYKVRGEKRVGVASSSHSKAGETIRPL
(D8A mutation shown in single underline,
H582 shown in bold and underlining)

Nuclear Localization Sequence (NLS)

[0050] The term "nuclear localization sequence" or "NLS" refers to an amino acid sequence that promotes import of a protein into the cell nucleus, for example, by nuclear transport. Nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank et al., International PCT application, PCT/EP2000/011690, filed Nov. 23, 2000, published as WO/2001/038547 on May 31, 2001, the contents of which are incorporated herein by reference for its disclosure of exemplary nuclear localization sequences. In some embodiments, a NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 75), MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 76), KRTADGSEFESPKKKRKV (SEQ ID NO: 374), or KRTADGSEFEPKKKRKV (SEQ ID NO: 375).

Nucleic Acid Molecule

[0051] The terms "nucleic acid" and "nucleic acid molecule," as used herein, refer to a compound comprising a nucleobase and an acidic moiety, e.g., a nucleoside, a nucleotide, or a polymer of nucleotides. Typically, polymeric nucleic acids, e.g., nucleic acid molecules comprising three or more nucleotides are linear molecules, in which adjacent nucleotides are linked to each other via a phosphodiester linkage. In some embodiments, "nucleic acid" refers to individual nucleic acid residues (e.g. nucleotides and/or nucleosides). In some embodiments, "nucleic acid" refers to an oligonucleotide chain comprising three or more individual nucleotide residues. As used herein, the terms "oligonucleotide" and "polynucleotide" can be used interchangeably to refer to a polymer of nucleotides (e.g., a string of at least three nucleotides). In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA. Nucleic acids may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a non-naturally occurring molecule, e.g., a recombinant DNA or RNA, an artificial chromosome, an engineered genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, e.g., analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (e.g. adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O (6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages).

Nucleic Acid Effector Domain

[0052] The term "nucleic acid effector domain," as used herein (or "nucleic acid editing domain") refers to a protein or enzyme capable of making one or more modifications (e.g., deamination of a cytidine residue) to a nucleic acid (e.g., DNA or RNA) or a protein associated with a nucleic acid (e.g., a histone protein). Exemplary nucleic acid editing domains include, but are not limited to a deaminase, a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain. In some embodiments the nucleic acid editing domain is a protein or enzyme capable of making one or more modifications (e.g., deamination of a cytidine residue) to a nucleic acid (e.g., DNA or RNA). In some embodiments the nucleic acid editing domain is a deaminase (e.g., a cytidine deaminase, such as an APOBEC or an AID deaminase).

Oligonucleotide

[0053] As used herein, the terms "oligonucleotide" and "polynucleotide" can be used interchangeably to refer to a polymer of nucleotides (e.g., a string of at least three nucleotides). In some embodiments, "nucleic acid" encompasses RNA as well as single and/or double-stranded DNA. Nucleic acids may be naturally occurring, for example, in the context of a genome, a transcript, an mRNA, tRNA, rRNA, siRNA, snRNA, a plasmid, cosmid, chromosome, chromatid, or other naturally occurring nucleic acid molecule. On the other hand, a nucleic acid molecule may be a

non-naturally occurring molecule, e.g., a recombinant DNA or RNA, an artificial chromosome, an engineered genome, or fragment thereof, or a synthetic DNA, RNA, DNA/RNA hybrid, or including non-naturally occurring nucleotides or nucleosides. Furthermore, the terms "nucleic acid," "DNA," "RNA," and/or similar terms include nucleic acid analogs, e.g., analogs having other than a phosphodiester backbone. Nucleic acids can be purified from natural sources, produced using recombinant expression systems and optionally purified, chemically synthesized, etc. Where appropriate, e.g., in the case of chemically synthesized molecules, nucleic acids can comprise nucleoside analogs such as analogs having chemically modified bases or sugars, and backbone modifications. A nucleic acid sequence is presented in the 5' to 3' direction unless otherwise indicated. In some embodiments, a nucleic acid is or comprises natural nucleosides (e.g. adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine); nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O (6)-methylguanine, and 2-thiocytidine); chemically modified bases; biologically modified bases (e.g., methylated bases); intercalated bases; modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose); and/or modified phosphate groups (e.g., phosphorothioates and 5'-N-phosphoramidite linkages).

Pharmaceutical Composition

[0054] The term "pharmaceutical composition," as used herein, refers to a composition that can be administrated to a subject in the context of treatment of a disease or disorder. In some embodiments, a pharmaceutical composition comprises an active ingredient, e.g., a base editor or a nucleic acid encoding a base editor, and a pharmaceutically acceptable excipient.

Proliferative Disease

[0055] The term "proliferative disease," as used herein, refers to any disease in which cell or tissue homeostasis is disturbed in that a cell or cell population exhibits an abnormally elevated proliferation rate. Proliferative diseases include hyperproliferative diseases, such as pre-neoplastic hyperplastic conditions and neoplastic diseases. Neoplastic diseases are characterized by an abnormal proliferation of cells and include both benign and malignant neoplasias. Malignant neoplasia is also referred to as cancer.

Promoter

[0056] The term "promoter" is art-recognized and refers to a nucleic acid molecule with a sequence recognized by the cellular transcription machinery and able to initiate transcription of a downstream gene. A promoter can be constitutively active, meaning that the promoter is always active in a given cellular context, or conditionally active, meaning that the promoter is only active in the presence of a specific condition. For example, a conditional promoter may only be active in the presence of a specific protein that connects a protein associated with a regulatory element in the promoter to the basic transcriptional machinery, or only in the absence of an inhibitory molecule. A subclass of conditionally active promoters are inducible promoters that require the presence of a small molecule "inducer" for activity. Examples of inducible promoters include, but are not limited to, arabinose-inducible promoters, Tet-on promoters, and tamoxifen-inducible promoters. A variety of constitutive, conditional, and inducible promoters are well known to the skilled artisan, and the skilled artisan will be able to ascertain a variety of such promoters useful in carrying out the instant invention, which is not limited in this respect.

Protein, Peptide, Polypeptide

[0057] The terms "protein," "peptide," and "polypeptide" are used interchangeably herein, and refer to a polymer of amino acid residues linked together by peptide (amide) bonds. The terms refer to a protein, peptide, or polypeptide of any size, structure, or function. Typically, a protein, peptide, or polypeptide will be at least three amino acids long. A protein, peptide, or polypeptide may refer to an individual protein or a collection of proteins. One or more of the amino acids in a protein, peptide, or polypeptide may be modified, for example, by the addition of a chemical entity such as a carbohydrate group, a hydroxyl group, a phosphate group, a farnesyl group, an isofarnesyl group, a fatty acid group, a linker for conjugation, functionalization, or other modification, etc. A protein, peptide, or polypeptide may also be a single molecule or may be a multi-molecular complex. A protein, peptide, or polypeptide may be just a fragment of a naturally occurring protein or peptide. A protein, peptide, or polypeptide may be naturally occurring, recombinant, or synthetic, or any combination thereof. The term "fusion protein" as used herein refers to a hybrid polypeptide which comprises protein domains from at least two different proteins. One protein may be located at the amino-terminal (N-terminal) portion of the fusion protein or at the carboxy-terminal (C-terminal) protein thus forming an "amino-terminal fusion protein" or a "carboxy-terminal fusion protein," respectively. A protein may comprise different domains, for example, a nucleic acid binding domain (e.g., the gRNA binding domain of Cas9 that directs the binding of the protein to a target site) and a nucleic acid cleavage domain or a catalytic domain of a nucleic-acid editing protein. In some embodiments, a protein comprises a proteinaceous part, e.g., an amino acid sequence constituting a nucleic acid binding domain, and an organic compound, e.g., a compound that can act as a nucleic acid cleavage agent. In some embodiments, a protein is in a complex with, or is in association with, a nucleic acid, e.g., RNA. Any of the proteins provided herein may be produced by any method known in the art. For example, the proteins provided herein may be produced via recombinant protein expression and purification, which is especially suited for fusion proteins comprising a peptide linker. Methods for recombinant protein expression and purification are well known, and include those described by Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (4th ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2012)), the entire contents of which are incorporated herein by reference.

Protospacer

[0058] As used herein, the term "protospacer" refers to the sequence (~20 bp) in DNA adjacent to the PAM (protospacer

adjacent motif) sequence which is complementary to the spacer sequence of the guide RNA. The guide RNA anneals to the protospacer sequence on the target DNA (specifically, one strand thereof, i.e, the "target strand" versus the "non-target strand" of the target sequence). In order for Cas9 to function it also requires a specific protospacer adjacent motif (PAM) that varies depending on the bacterial species of the Cas9 gene. The most commonly used Cas9 nuclease, derived from *S. pyogenes*, recognizes a PAM sequence of NGG that is found directly downstream of the target sequence in the genomic DNA, on the non-target strand. The skilled person will appreciate that the literature in the state of the art sometimes refers to the "protospacer" as the ~20-nt target-specific guide sequence on the guide RNA itself, rather than referring to it as a "spacer." Thus, in some cases, the term "protospacer" as used herein may be used interchangeably with the term "spacer." The context of the discription surrounding the appearance of either "protospacer" or "spacer" will help inform the reader as to whether the term is refence to the gRNA or the DNA target. Both usages of these terms are acceptable since the state of the art uses both terms in each of these ways.

Protospacer Adjacent Motif (PAM)

[0059] As used herein, the term "protospacer adjacent sequence" or "PAM" refers to an approximately 2-6 base pair DNA sequence (or a 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-long nucleotide sequence) that is an important targeting component of a Cas9 nuclease. Typically, the PAM sequence is on either strand, and is downstream in the 5' to 3' direction of Cas9 cut site. The canonical PAM sequence (i.e., the PAM sequence that is associated with the Cas9 nuclease of *Streptococcus pyogenes* or SpCas9) is 5'-NGG-3' wherein "N" is any nucleobase followed by two guanine ("G") nucleobases. Different PAM sequences can be associated with different Cas9 nucleases or equivalent proteins from different organisms. In addition, any given Cas9 nuclease, e.g., GeoCas9, may be modified to alter the PAM specificity of the nuclease such that the nuclease recognizes alternative PAM sequence.

[0060] For example, with reference to the canonical SpCas9 amino acid sequence is SEQ ID NO: 1, the PAM sequence can be modified by introducing one or more mutations, including (a) D1135V, R1335Q, and T1337R "the VQR variant", which alters the PAM specificity to NGAN or NGNG, (b) D1135E, R1335Q, and T1337R "the EQR variant", which alters the PAM specificity to NGAG, and (c) D1135V, G1218R, R1335E, and T1337R "the VRER variant", which alters the PAM specificity to NGCG. In addition, the D1135E variant of canonical SpCas9 still recognizes NGG, but it is more selective compared to the wild type SpCas9 protein. The corresponding or equivalent amino acid positions in any GeoCas9 amino acid sequence could be altered similary to achieve an altered PAM specificity (e.g., by altering SEQ ID NOs: 2, 3, or 5-8).

[0061] It will also be appreciated that Cas9 enzymes from different bacterial species (i.e., Cas9 orthologs) can have varying PAM specificities. For example, Cas9 from *Staphylococcus aureus* (SaCas9) recognizes NGRRT or NGRRN. In addition, Cas9 from *Neisseria meningitis* (NmCas) recognizes NNNNGATT. In another example, Cas9 from *Streptococcus thermophilis* (StCas9) recognizes NNAGAAW. In still another example, Cas9 from *Treponema denticola* (TdCas) recognizes NAAAAC. These are example are not meant to be limiting. It will be further appreciated that non-SpCas9s bind a variety of PAM sequences, which makes them useful when no suitable SpCas9 PAM sequence is present at the desired target cut site. Furthermore, non-SpCas9s may have other characteristics that make them more useful than SpCas9. For example, Cas9 from *Staphylococcus aureus* (SaCas9) is about 1 kilobase smaller than SpCas9, so it can be packaged into adeno-associated virus (AAV). Further reference may be made to Shah et al., "Protospacer recognition motifs: mixed identities and functional diversity," RNA Biology, 10 (5): 891-899 (which is incorporated herein by reference). The corresponding or equivalent amino acid positions in any GeoCas9 amino acid sequence could be altered similary to achieve an altered PAM specificity (e.g., by altering SEQ ID NOs: 2, 3, or 5-8).

Recombinant

[0062] The term "recombinant" as used herein in the context of proteins or nucleic acids refers to proteins or nucleic acids that do not occur in nature, but are the product of human engineering. For example, in some embodiments, a recombinant protein or nucleic acid molecule comprises an amino acid or nucleotide sequence that comprises at least one, at least two, at least three, at least four, at least five, at least six, or at least seven mutations as compared to any naturally occurring sequence.

Recombinase

[0063] The term "recombinase," as used herein, refers to a site-specific enzyme that mediates the recombination of DNA between recombinase recognition sequences, which results in the excision, integration, inversion, or exchange (e.g., translocation) of DNA fragments between the recombinase recognition sequences. Recombinases can be classified into two distinct families: serine recombinases (e.g., resolvases and invertases) and tyrosine recombinases (e.g., integrases). Examples of serine recombinases include, without limitation, Hin, Gin, Tn3, β-six, CinH, ParA, γδ, Bxb1, ϕC31, TP901, TG1, φBT1, R4, φRV1, φFC1, MR11, A118, U153, and gp29. Examples of tyrosine recombinases include, without limitation, Cre, FLP, R, Lambda, HK101, HK022, and pSAM2. The Gin recombinase referred to herein may be any Gin recombinase known in the art including, but not limited to, the Gin recombinases presented in T. Gaj et al., A comprehensive approach to zinc-finger recombinase customization enables genomic targeting in human cells. Nucleic Acids Research 41, 3937-3946 (2013), incorporated herein by reference in its entirety. Exemplary recombinase sequences are disclosed herein.

RNA-Guided Protein

[0064] The term "RNA-guided protein" refers to a protein (e.g., an RNA-guided nuclease) that forms a complex with (e.g., binds or associates with) one or more RNA(s) that is not a target for cleavage. In some embodiments, an RNA-guided protein, when in a complex with an RNA, may be referred to as a RNA-guided-protein: RNA complex. In some embodiments, the RNA-guided protein: RNA complex binds to a target nucleic acid (e.g., DNA) sequence, such as a sequence within a genome of an organism, via complementary binding of a portion of the RNA to the nucleic acid. Typically, the bound RNA(s) is referred to as a guide RNA (gRNA). gRNAs can exist as a complex of two or more

RNAs, or as a single RNA molecule. gRNAs that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), though "gRNA" is also used to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as a single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (i.e., directs binding of a Cas9 complex to the target); and (2) a domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA and comprises a stem-loop structure. In some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek et al., *Science* 337:816-821 (2012), the entire contents of which are incorporated herein by reference. Other examples of gRNAs (e.g., those including domain 2) can be found in U.S. Provisional Patent Application, U.S. Ser. No. 61/874,682, filed Sep. 6, 2013, entitled "Switchable Cas9 Nucleases And Uses Thereof," and U.S. Provisional Patent Application, U.S. Ser. No. 61/874,746, filed Sep. 6, 2013, entitled "Delivery System For Functional Nucleases," the entire contents of each are hereby incorporated by reference in their entirety. In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an "extended gRNA." For example, an extended gRNA will bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease: RNA complex.

[0065] In some embodiments, the gRNA backbone sequence is a gRNA backbone sequence that binds to a GeoCas9 protein. gRNA backbone sequences that bind to GeoCas9 are known in the art and have been described previously, for example, in Harrington, L. B. et al., A thermostable Cas9 with increased lifetime in human plasma" Nat. Commun., 2017 Nov. 10; 8 (1): 1424. The entire contents of which are incorporated herein by reference. In some embodiments, the gRNA sequence comprises the nucleic acid sequence 5'-GUCAUAGUUCCC-CUGAGAAAUCAGGGUUACUAUGAUAAGGGC-UUUCUGCCUA AGGCA-GACUGACCCGCGGCGUUGGGGAUCGCCUGUCGC CCGCUUUUGGCGGGC AUUCCCCAUCCUUUUUUU-3' (SEQ ID NO: 77), or a variant thereof that binds to a GeoCas9. For example, the gRNA may comprise a nucleic acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleic acid changes relative to (SEQ ID NO: 77) and is capable of binding to a GeoCas9. In some embodiments, the gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease: RNA complex. In some embodiments, the nucleotide sequence that complements a target site, comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 contiguous nucleotides that are complementary to a target site.

[0066] For example the gRNAsequence may comprise the structure 5'-[nucleotide sequence that complements a target site]-GUCAUAGUUCCCCUGAGAAAUCAGGGUUAC-UAUGAUAAGGGCUUUCUGCCUA AGGCA-GACUGACCCGCGGCGUUGGGGAUCGCCUGU-CGCCCGCUUUUGGCGGGC AUUCCCCAUCCUUUUUUU-3' (SEQ ID NO: 77). In some embodiments, the nucleotide sequence that complements a target site comprises 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 contiguous nucleotides. In some embodiments, the RNA-guided protein is a nuclease, such as a Cas9 (e.g., GeoCas9) that cleaves one or both strands of a duplexed DNA molecule. In some embodiments, the Cas9 is a fully active GeoCas9 that cleaves both strands of a duplexed DNA molecule. In some embodiments, the Cas9 is a GeoCas9 nickase (GeoCas9n) tha cleaves one strand of a duplexed DNA molecule. In some embodiments, the RNA-guided protein is a GeoCas9 that has been engineered (e.g., mutated) to silence nuclease activity.

[0067] Because RNA-guided nucleases (e.g., Cas9) use RNA: DNA hybridization to target DNA cleavage sites, these proteins are able to target, in principle, any sequence specified by the guide RNA. Methods of using RNA-guided nucleases, such as Cas9, for site-specific cleavage (e.g., to modify a genome) are known in the art (see e.g., Cong, L. et al., Multiplex genome engineering using CRISPR/Cas systems. *Science* 339, 819-823 (2013); Mali, P. et al., RNA-guided human genome engineering via Cas9. *Science* 339, 823-826 (2013); Hwang, W. Y. et al., Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature biotechnology* 31, 227-229 (2013); Jinek, M. et al. RNA-programmed genome editing in human cells. *eLife* 2, e00471 (2013); Dicarlo, J. E. et al., Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Research* (2013); Jiang, W. et al., RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology* 31, 233-239 (2013); the entire contents of each of which are incorporated herein by reference).

Spacer Sequence

[0068] As used herein, the term "spacer sequence" in connection with a guide RNA refers to the portion of the guide RNA of about 20 nucleotides (or from about 5, 6, 7, 8, 9, 11, 12 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, or more) which contains a nucleotide sequence that is complementary to the protospacer sequence in the target DNA sequence. The spacer sequence anneals to the protospacer sequence to form a ssRNA/ssDNA hybrid structure at the target site and a corresponding R loop ssDNA structure of the endogenous DNA strand that is complementary to the protospacer sequence.

Subject

[0069] The term "subject," as used herein, refers to an individual organism, for example, an individual mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal. In some embodiments, the subject is a non-human primate. In some embodiments, the subject is a rodent. In some embodiments, the subject is a sheep, a goat, a cattle, a cat, or a dog. In some embodiments, the subject is a vertebrate, an amphibian, a reptile, a fish, an insect, a fly, or a nematode. In some embodiments, the subject is a research animal. In some embodiments, the subject is genetically engineered, e.g., a genetically engineered non-human subject. The subject may

be of either sex and at any stage of development. In some embodiments, the subject is a healthy volunteer.

Target Site

[0070] The term "target site" refers to a nucleobase or a sequence within a nucleic acid molecule that is targeted and/or modified by a nucleic acid editing domain. In some embodiments, a target site refers to a cytidine within a nucleic acid molecule that is deaminated by a deaminase or a fusion protein comprising a deaminase, (e.g., a GeoCas9-deaminase fusion protein provided herein).

Transitions

[0071] As used herein, "transitions" refer to the interchange of purine nucleobases (A↔G) or the interchange of pyrimidine nucleobases (C↔T). This class of interchanges involves nucleobases of similar shape. The compositions and methods disclosed herein are capable of inducing one or more transitions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule. These changes involve A↔G, G↔A, C↔T, or T↔C. In the context of a double-strand DNA with Watson-Crick paired nucleobases, transversions refer to the following base pair exchanges: A:T↔G:C, G:G↔A:T, C:G↔T:A, or T:A↔C:G. The compositions and methods disclosed herein are capable of inducing one or more transitions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule, as well as other nucleotide changes, including deletions and insertions.

Transversions

[0072] As used herein, "transversions" refer to the interchange of purine nucleobases for pyrimidine nucleobases, or in the reverse and thus, involve the interchange of nucleobases with dissimilar shape. These changes involve T↔A, T↔G, C↔G, C↔A, A↔T, A↔C, G↔C, and G↔T. In the context of a double-strand DNA with Watson-Crick paired nucleobases, transversions refer to the following base pair exchanges: T:A↔A:T, T:A↔G:C, C:G↔G:C, C:G↔A:T, A:T↔T:A, A:T↔C:G, G:C↔C:G, and G:C↔T:A. The compositions and methods disclosed herein are capable of inducing one or more transversions in a target DNA molecule. The compositions and methods disclosed herein are also capable of inducing both transitions and transversion in the same target DNA molecule, as well as other nucleotide changes, including deletions and insertions.

Treatment

[0073] The terms "treatment," "treat," and "treating" refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. As used herein, the terms "treatment," "treat," and "treating" refer to a clinical intervention aimed to reverse, alleviate, delay the onset of, or inhibit the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed and/or after a disease has been diagnosed. In other embodiments, treatment may be administered in the absence of symptoms, e.g., to prevent or delay onset of a symptom or inhibit onset or progression of a disease. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors). Treatment may also be continued after symptoms have resolved, for example, to prevent or delay their recurrence.

Uracil Glycosylase Inhibitor

[0074] The term "uracil glycosylase inhibitor" or "UGI," as used herein, refers to a protein that is capable of inhibiting a uracil-DNA glycosylase base-excision repair enzyme. In some embodiments, a UGI domain comprises a wild-type UGI or a UGI as set forth in SEQ ID NO: 60-74. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. For example, in some embodiments, a UGI domain comprises a fragment of the amino acid sequence set forth in SEQ ID NO: 60-74. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid sequence as set forth in SEQ ID NO: 60-74. In some embodiments, a UGI comprises an amino acid sequence homologous to the amino acid sequence set forth in SEQ ID NO: 60-74, or an amino acid sequence homologous to a fragment of the amino acid sequence set forth in SEQ ID NO: 60-74. In some embodiments, proteins comprising UGI or fragments of UGI or homologs of UGI or UGI fragments are referred to as "UGI variants." A UGI variant shares homology to UGI, or a fragment thereof. For example a UGI variant is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to a wild type UGI or a UGI as set forth in SEQ ID NO: 60-74. In some embodiments, the UGI variant comprises a fragment of UGI, such that the fragment is at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% to the corresponding fragment of wild-type UGI or a UGI as set forth in SEQ ID NO: 60-74.

[0075] In some embodiments, the UGI comprises the amino acid sequence of SEQ ID NO: 60, as set forth below. Exemplary Uracil-DNA glycosylase inhibitor (UGI;

```
>sp|P14739|UNGI_BPPB2)
                                        (SEQ ID NO: 60)
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDES

TDENVMLLTSDAPEYKPWALVIQDSNGENKIKML.
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DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[0076] Some aspects of this disclosure provide fusion proteins that comprise a domain capable of binding to a nucleotide sequence (e.g., a GeoCas9 protein) and a nucleic acid effector domain, for example, a DNA-editing domain, such as a deaminase domain, recominase domain, or meth-

yltransferase domain. All such fusion proteins may be referred to herein as "GeoCas9 base editors" or "GeoCas9 base editor fusion proteins." In some cases, these proteins may be referred to simply as "fusion proteins."

[0077] For example, in the case where the nucleic acid effector domain is a deaminase, the deamination of a nucleobase by a deaminase can lead to a point mutation at the respective residue, which is referred to herein as nucleic acid editing or "base editing." Fusion proteins comprising a GeoCas9 variant and a DNA editing domain can thus be used for the targeted editing of nucleic acid sequences.

[0078] Such GeoCas9 base editor fusion proteins are useful for targeted editing of DNA in vitro, e.g., for the generation of mutant cells or animals; for the introduction of targeted mutations, e.g., for the correction of genetic defects in cells ex vivo, e.g., in cells obtained from a subject that are subsequently re-introduced into the same or another subject; and for the introduction of targeted mutations, e.g., the correction of genetic defects or the introduction of deactivating mutations in disease-associated genes in a subject. Typically, the GeoCas9 of the fusion proteins described herein does not have full nuclease activity but instead is a dGeoCas9 protein or a GeoCas9 nickase (GeoCas9n). Other aspects of the invention provide fusion proteins that comprise (i) a domain capable of binding to a nucleic acid sequence (e.g., a GeoCas9 or fragment thereof) and (ii) an enzyme domain, for example, a DNA-editing domain (e.g., a deaminase domain). In various embodiments, the GeoCas9 base editor fusion proteins may also comprise (iii) one or more uracil glycosylase inhibitor (UGI) domains. The presence of at least one UGI domain increases base editing efficiency compared to fusion proteins without a UGI domain. In some embodiments, a fusion protein comprising two UGI domains further increases base editing efficiency and product purity compared to fusion proteins with one UGI domain or without a UGI domain. Methods for the use of GeoCas9 fusion proteins as described herein are also provided.

I. GeoCas9 Base Editors

[0079] In various aspects, the disclosure provides GeoCas9 base editor fusion proteins for use in base editing of target DNA molecule (e.g., a genome). In various embodiments, the GeoCas9 base editor fusion proteins are complexed with a guide RNA that programs the GeoCas9 base editor to target a specific nucleotide sequence. The various components of the GeoCas9 base editor fusion proteins (e.g., the GeoCas9 domain and the one or more effector domains) and the guide RNAs are described further herein. In various embodiments, the GeoCas9 base editor fusion proteins comprises a GeoCas9 domain and an effector domain, wherein the effector domain can include, but are not limited to, a deaminase (e.g., a cytidine deaminase or an adenosine deaminase), a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain.

A. GeoCas9 Proteins

[0080] The GeoCas9 base editors may comprise any suitable GeoCas9 protein. The GeoCas9 proteins may be a naturally occurring GeoCas9 protein. The GeoCas9 may also be a variant of a naturally occurring GeoCas9 protein. Such variants may comprise one or more mutations. Such mutations may be introduced using recombinant techniques, mutagenesis, or sequence evolution techniques (e.g., PACE or PANCE). Variants may also include GeoCas9 fragments which main some of all of the function of a complete GeoCas9 protein. Such variants are described further herein. In some embodiments, such variants may be those sequences with are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% similar to any of the GeoCas9 sequences provided herein.

[0081] Non-limiting, exemplary GeoCas9 proteins are provided herein. The GeoCas9 may be a nuclease active GeoCas9, a nucleasae inactive GeoCas9 (dGeoCas9), or a GeoCas9 nickase (GeoCas9n). In some embodiments, the GeoCas9 is a nuclease active GeoCas9. For example, the GeoCas9 may be a GeoCas9 that cuts both strands of a duplexed nucleic acid (e.g., both strands of a duplexed DNA molecule). In some embodiments the GeoCas9 comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any naturally-occurring GeoCas9 to any one of the amino acid sequences set forth in SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to any naturally-occurring GeoCas9 or to any one of the amino acid sequences set forth in SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at leat 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1050, at least 1060, at least 1070, or at least 1080 identical contiguous amino acid residues as compared to any naturally-occurring GeoCas9 or to any one of the amino acid sequences set forth in SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 comprises any one of the amino acid sequences as set forth in SEQ ID NOs: 2, 3, or 5-8.

[0082] In some embodiments, the GeoCas9 is a nuclease-inactive GeoCas9 (dGeoCas9). For example, the dGeoCas9 may bind to a duplexed nucleic acid molecule (e.g., via a gRNA molecule) without cleaving either strand of the duplexed nucleic acid molecule. In some embodiments, the nuclease-inactive dGeoCas9 comprises a $D7X_1$ mutation and a $H581X_2$ mutation of the amino acid sequence set forth in SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as any one of the amino acid sequences provided in SEQ ID NOs: 2, 3, or 5-8, where X_1 is any amino acid except D and where X_2 is any amino acid except H. In some embodiments, the nuclease-inactive dGeoCas9 comprises a D7A mutation and a H581A mutation of the amino acid sequence set forth in SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as any one of the amino acid sequences provided in SEQ ID NOs: 2, 3, or 5-8. As one example, a nuclease-inactive GeoCas9d comprises the amino acid sequence set forth in SEQ ID NO: 7.

[0083] Additional suitable nuclease-inactive dGeoCas9 proteins will be apparent to those of skill in the art based on

this disclosure and knowledge in the field, and are within the scope of this disclosure. Examples of additional nuclease-inactive GeoCas9 proteins include, but are not limited to, D8A/H582A, D8A/D581A/H582A, and D8A/D581A/H582A/N605A mutations of the amino acid sequence set forth in SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as any one of the amino acid sequences provided in SEQ ID NOs: 2, 3, or 5-8 (See, e.g., Prashant et al., CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. *Nature Biotechnology.* 2013; 31 (9): 833-838, the entire contents of which are incorporated herein by reference). In some embodiments the dGeoCas9 comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any of the GeoCas9 proteins provided herein, but is not capable of cleaving a strand of a nucleic acid (e.g., DNA or RNA) molecule. In some embodiments, the GeoCas9 comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more or more mutations compared to any naturally-occurring GeoCas9, or any one of the amino acid sequences set forth in SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 comprises an amino acid sequence that has at least 10, at least 15, at least 20, at least 30, at leat 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 200, at least 250, at least 300, at least 350, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1050, at least 1060, at least 1070, or at least 1080 identical contiguous amino acid residues as compared to any GeoCas9, such as any one of the amino acid sequences set forth in SEQ ID NOs: 2, 3, or 5-8.

[0084] In some embodiments, the GeoCas9 is a GeoCas9 nickase (GeoCas9n). The GeoCas9 nickase may be a GeoCas9 protein that is capable of cleaving only one strand of a duplexed nucleic acid molecule (e.g., a duplexed DNA molecule). In some embodiments the GeoCas9 nickase cleaves the target strand of a duplexed nucleic acid molecule, meaning that the GeoCas9 nickase cleaves the strand that is base paired to (complementary to) a gRNA (e.g., an sgRNA) that is bound to the GeoCas9 nickase. In some embodiments, a GeoCas9 nickase comprises a D7A mutation and has a histidine at position 581 of SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as any one of the GeoCas9 proteins provided in SEQ ID NOs: 2, 3, or 5-8. For example, a GeoCas9 nickase may comprise the amino acid sequence as set forth in SEQ ID NO: 2. In some embodiments the GeoCas9 nickase cleaves the non-target, non-base-edited strand of a duplexed nucleic acid molecule, meaning that the GeoCas9 nickase cleaves the strand that is not base paired to a gRNA (e.g., a sgRNA) that is bound to the Cas9. In some embodiments, a GeoCas9 nickase comprises an H581A mutation and has an aspartic acid residue at position 7 of SEQ ID NO: 3, or a corresponding mutation in any GeoCas9, such as the GeoCas9 of any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments the Cas9 nickase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any GeoCas9, such as the GeoCas9 of any one of SEQ ID NOs: 2, 3, or 5-8, and is capable of cleaving one, but not both, strands of a duplexed nucleic acid (e.g., DNA or RNA) molecule. Additional suitable Cas9 nickases will be apparent to those of skill in the art based on this disclosure and knowledge in the field, and are within the scope of this disclosure.

[0085] Exemplary GeoCas9 sequences for use in the this disclosure are as follows:

dGeoCas9 (D8A and H582A):

(SEQ ID NO: 7)

MKYKIGL**A**IGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLAR

SARRRLRRRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNN

DELARILLHLAKRRGFRSNRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDP

KFSLHKRNKEDNYTNTVARDDLEREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRP

FASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFTVWEHINKLRLVSPGGIRALTDDER

RLIYKQAFHKNKITFHDVRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFLELGAYHK

IRKAIDSVYGKGAAKSFRPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLA

DKVYDEELIEELLMLSFSKFGHLSLKALRNILPYMEQGEVYSTACERAGYTFTGPKKK

QKTVLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARELSQSFDERRKM

QKEQEGNRKKNETAIRQLVEYGLTLNPTGLDIVKFKLWSEQNGKCAYSLQPIEIERLL

EPGYTEVD**A**VIPYSRSLDDSYTNKVLVLTKENREKGNRTPAEYLGLGSERWQQFETF

VLTNKQFSKKKRDRLLRLHYDENEENEFKNRNLNDTRYISRFLANFIREHLKFADSD

DKQKVYTVNGRITAHLRSRWNFNKNREESNLHHAVDAAIVACTTSDIARVTAFYQ

RREQNKELSKKTDPQFPQPWPHFADELQARLSKNPKESIKALNLGNYDNEKLESLQP

VFVSRMPKRSITGAAHQETLRRYIGIDERSGKIQTVVKKKLSEIQLDKTGHFPMYGKE

-continued

SDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGELGPIIRTIKIIDTTNQVIPLNDGK

TVAYNSNIVRRVDVFEKDGKYYCVPIYTIDMMKGILPNKAIEPNKPYSEWKEMTEDYT

FRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYYQTIDSSNGGLSLVSHDNNFSLR

SIGSRTLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHSKAGETIRPL
(D8A and H582A mutations shown in bold and underlining)

GeoCas9 nickase (D8A) - Used in GeoBE3 and GeoBE4 Constructs
(SEQ ID NO: 2)

RYKIGLAIGITSVGWAVMNLDIPRIEDLGVRIFDRAENPQTGESLALPRRLAR

SARRRLRRRKHRLERIRRLVIREGILTKEELDKLFEEKHIEDVWQLRVEALDRKLNND

ELARVLLHLAKRRGFKSMRKSERSNKENSTMLKHIEENRAILSSYRTVGEMIVKDPK

FALHKRNKGENYTNTIARDDLEREIRLIFSKQREFGNMSCTEEFENEYITIWASQRPV

ASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIAWEHINKLRLISPSGARGLTDEERRL

LYEQAFQKNKITYHDIRTLLHLPDDTYFKGIVYDRGESRKQNENIRFLELDAYHQIRK

AVDKVYGKGKSSSFLPIDFDTFGYALTLFKDDADIHSYLRNEYEQNGKRMPMLALK

VYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEVYSSACERAGYTFTGPKKKQK

TMPLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARDLSQTFDERRKTKK

EQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQNGRCAYSLQPIEIERLLEP

GYVEVDHVIPYSRSLDDSYTNKVLVLTRENREKGNRIPAEYLGVGTERWQQFETFVL

TNKQFSKKKDRLLRLHYDENEETEFKNRNLNFTRYISRFFANFIREHLKFAESDDK

QKVYTVNGRVTAHLRSRWEFNKNREESDLHHAVDAVIVACTTPSDIAKVTAFYQRR

EQNKELAKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGNYDDQKLESLQPVF

VSRMPKRSVTGAAHQETLRRYVGIDERSGKIQTVVKTKLSEIKLDASGHFPMYGKES

DPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVKIIDTKNQVIPLNDG

KTVAYNSNIVRVDVFEKDGKYYCVPVYTMDIMKGILPNKAIEPNKPYSEWKEMTED

YTFRFSLYPNDLIRIELPREKTVKTAAGEEINVKDVFVYYKTIDSANGGLELISHDHRF

SLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVGLASSAHSKPGKTIRPLQSTRD

GeoCas9 wt - Wild type version of the nickase used in GeoBE3
and GeoBE4 Consturcts
(SEQ ID NO: 3)

RYKIGLDIGITSVGWAVMNLDIPRIEDLGVRIFDRAENPQTGESLALPRRLAR

SARRRLRRRKHRLERIRRLVIREGILTKEELDKLFEEKHEIDVWQLRVEALDRKLNND

ELARVLLHLAKRRGFKSNRKSERSNKENSTMLKHIEENRAILSSYRTVGEMIVKDPK

FALHKRNKGENYTNTIARDDLEREIRLIFSKQREFGNMSCTEEFENEYITIWASQRPV

ASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIAWEHINKLRLISPSGARGLTDEERRL

LYEQAGQKNKITYHDIRTLLHLPDDTYFKGIVYDRGESRKQNENIRFLELDAYHQIRK

AVDKVYGKGKSSSFLPIDFDTFGYALTLFKDDADIHSYLRNEYEQNGKRMPNLANK

VYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEVYSSACERAGYTFTGPKKKQK

TMLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARDLSQTFDERRKTKK

EQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQNGRCAYSLQPIEIERLLEP

GYVEVDHVIPYSRSLDDSYTNKVLVLTRENREKGNRIPAEYLGVGTERWQQFETFVL

TNKQFSKKKRDRLLRLHYDENEETEFKNRNLNDTRYISRFFANFIREHLKFAESDDK

QKVYTVNGRVTAHLRSRWEFNKNREESDLHHAVDAVIVACTTPSDIAKVTAFYQRR

-continued

EQNKELAKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGNYDDQKLESLQPVF

VSRMPKRSVTGAAHQETLRRYVGIDERSGKIQTVVKTKLSEIKLDASGHFPMYGKES

DPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVKIIDTKNQVIPLNDG

KTVAYNSNIVRVDVFEKDGKYYCVPVYTMDIMKGILPNKAIEPNKPYSEWKEMTED

YTFRFSLYPNDLIRIELPREKTVKTAAGEEINVKDVFVYYKTIDSANGGLELISHDHRF

SLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVGLASSAHSKPGKTIRPLQSTRD

In some embodiements, GeoCas9 corresponds to Cas9 from *Geobacillus stearothermophilus* (>tr|A0A178TEJ9|A0A178TEJ9_GEOSE CRISPR-associated endonuclease Cas9 OS = *Geobacillus stearothermophilus* GN = cas9 PE = 3 SV = 1; SEQ ID NO: 6)

(SEQ ID NO: 6)

MRYKIGLDIGITSVGWAVMNLDIPRIEDLGVRIFDRAENPQTGESLALPRRLA

RSARRRLRRRKHRLERIRRLVIREGILTKEELDKLFEEKHEIDVWQLRVEALDRKLNN

DELARVLLHLAKRRGFKSNRKSERSNKENSTMLKHIEENRAILSSYRTVGEMIVKDP

KFALHKRNKGENYTNTIARDDLEREIRLIFSKQREFGNMSCTEEFENEYITIWASQRP

VASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIAWEHINKLRLISPSGARGLTDEER

RLLYEQAFQKNKITYHDIRTLLHLPDDTYFKGIVYDRGESRKQNENIRFLELDAYHQI

RKAVDKVYGKGKSSSFLPIDFDTFGYALTLFKDDADIHSYLRNEYEQNGKRMPNLA

NKVYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEVYSSACERAGYTFTGPKKK

QKTMLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARDLSQTFDERRKT

KKEQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQNGRCAYSLQPIEIERL

LEPGYVEVDHVIPYSRSLDDSYTNKVLVLTRENREKGNRIPAEYLGVGTERWQQFET

FVLTNKQFSKKKRDRLLRLHYDENEETEFKNRNLNDTRYISRFFANFIREHLKFAESD

DKQKVYTVNGRVTAHLRSRWEFNKNREESDLHHAVDAAIVACTTPSDIAKVTAFYQ

RREQNKELAKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGNYDDQKLESLQP

VFVSRMPKRSVTGAAHQETLRRYVGIDERSGKIQTVVKTKLSEIKLDASGHFPMYGK

ESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVKIIDTKNQVIPLND

GKTVAYNSNIVRVDVFEKDGKYYCVPVYTMDIMKGILPNKAIEPNKPYSEWKEMTE

DYTFRFSLYPNDLIRIELPREKTVKTAAGEEINVKDFVYYKTIDSANGGLELISHDHR

FSLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVGLASSAHSKTGETVRPLQSTRD

In some embodiments, GeoCas9 corresponds to Cas9 from *Geobacillus thermodenitrificans* (NCBI Reference Sequence: WP_087959824.1 SEQ ID NO: 4 (nucleotide); SEQ ID NO: 5 (amino acid)).

(SEQ ID NO: 4)

atgaagtataaaatcggtcttgctatcggcattacgtctatcggttgggctgtcattaatttggac

attcctcgcatcgaagatttaggtgtccgcatttttgacagagcggaaaacccgaaaaccggggag

tcactagctcttccacgtcgcctcgcccgctccgcccgacgtcgtctgcggcgtcgcaaacatcga

ctggagcgcattcgccgcctgttcgtccgcgaaggaattttaacgaaggaagagctgaacaagctg

tttgaaaaaaagcacgaaatcgacgtctggcagcttcgtgttgaagcactggatcgaaaactaaat

aacgatgaattagcccgcatccttcttcatctggctaaacggcgtggatttagatccaaccgcaag

agtgagcgcaccaacaaagaaaacagtacgatgctcaaacatattgaagaaaaccaatccattctt

tcaagttaccgaacggttgcagaaatggttgtcaaggatccgaaattttccctgcacaagcgtaat

aaagaggataattacaccaacactgttgcccgcgacgatcttgaacgggaaatcaaactgattttc

-continued

gccaaacagcgcgaatatgggaacatcgtttgcacagaagcatttgaacacgagtatatttccatt

tgggcatcgcaacgccctttttgcttctaaggatgatatcgagaaaaaagtcggtttctgtacgttt

gagcctaaagaaaaacgcgcgccaaaagcaacatacacattccagtccttcaccgtctgggaacat

attaacaaacttcgtcttgtctccccgggaggcatccgggcactaaccgatgatgaacgtcgtctt

atatacaagcaagcatttcataaaaataaaatcaccttccatgatgttcgaacattgcttaacttg

cctgacgacacccgttttaaaggtcttttatatgaccgaaacaccacgctgaaggaaaatgagaaa

gttcgcttccttgaactcggcgcctatcataaaatacggaaagcgatcgacagcgtctatggcaaa

ggagcagcaaaatcatttcgtccgattgattttgatacatttggctacgcattaacgatgtttaaa

gacgacaccgacattcgcagttacttgcgaaacgaatacgaacaaaatggaaaacgaatggaaaat

ctagcggataaagtctatgatgaagaattgattgaagaacttttaaacttatcgttttctaagttt

ggtcatctatcccttaaagcgcttcgcaacatccttccatatatggaacaaggcgaagtctactca

accgcttgtgaacgagcaggatatacatttacagggccaaagaaaaaacagaaaacggtattgctg

ccgaacattccgccgatcgccaatccggtcgtcatgcgcgcactgacacaggcacgcaaagtggtc

aatgccattatcaaaaagtacggctcaccggtctccatccatatcgaactggcccgggaactatca

caatcctttgatgaacgacgtaaaatgcagaaagaacaggaaggaaaccgaaagaaaaacgaaact

gccattcgccaacttgttgaatatgggctgacgctcaatccaactgggcttgacattgtgaaattc

aaactatggagcgaacaaaacggaaaatgtgcctattcactccaaccgatcgaaatcgagcggttg

ctcgaaccaggctatacagaagtcgacgctgtgattccatacagccgaagcttggacgatagctat

accaataaagttcttgtgttgacaaaggagaaccgtgaaaaaggaaaccgcaccccagctgaatat

ttaggattaggctcagaacgttggcaacagttcgagacgtttgtcttgacaaataagcagttttcg

aaaaagaagcgggatcgactccttcggcttcattacgatgaaaacgaagaaaatgagtttaaaaat

cgtaatctaaatgatacccgttatatctcacgcttcttggctaactttattcgcgaacatctcaaa

ttcgccgacagcgatgacaaacaaaaagtatacacggtcaacggccgtattaccgcccatttacgc

agccgttggaattttaacaaaaaccgggaagaatcgaatttgcatcatgccgtcgatgctgccatc

gtcgcctgcacaacgccgagcgatatcgcccgagtcaccgccttctatcaacggcgcgaacaaaac

aaagaactgtccaaaaagacggatccgcagtttccgcagccttggccgcactttgctgatgaactg

caggcgcgtttatcaaaaaatccaaaggagagtataaaagctctcaatcttggaaattatgataac

gagaaactcgaatcgttgcagccggtttttgtctcccgaatgccgaagcggagcataacaggagcg

gctcatcaagaaacattgcggcgttatatcggcatcgacgaacggagcggaaaaatacagacggtc

gtcaaaaagaaactatccgagatccaactggataaaacaggtcatttcccaatgtacgggaaagaa

agcgatccaaggacatatgaagccattcgccaacggttgcttgaacataacaatgacccaaaaaag

gcgtttcaagagcctctgtataaaccgaagaagaacggagaactaggtcctatcatccgaacaatc

aaaatcatcgatacgacaaatcaagttattccgctcaacgatggcaaaacagtcgcctacaacagc

aacatcgtgcgggtcgacgtctttgagaaagatggcaaatattattgtgtccctatctatacaata

gatatgatgaaagggatcttgccaaacaaggcgatcgagccgaacaaaccgtactctgagtggaag

-continued

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gaaatgacggaggactatacattccgattcagtctatacccaaatgatcttatccgtatcgaattt
ccccgagaaaaaacaataaagactgctgtgggggaagaaatcaaaattaaggatctgttcgcctat
tatcaaaccatcgactcctccaatggagggttaagtttggttagccatgataacaacttttcgctc
cgcagcatcggttcaagaaccctcaaacgattcgagaaataccaagtagatgtgctaggcaacatc
tacaaagtgagaggggaaaagagagttggggtggcgtcatcttctcattcgaaagccggggaaact
atccgtccgttataa
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(SEQ ID NO: 5)

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MKYKIGLDIGITSIGWAVINLDIPRIEDLGVRIFDRAENPKTGESLALPRRLAR
SARRRLRRRKHRLERIRRLFVREGILTKEELNKLFEKKHEIDVWQLRVEALDRKLNN
DELARILLHLAKRRGFRSNRKSERTNKENSTMLKHIEENQSILSSYRTVAEMVVKDP
KFSLHKRNKEDNYTNTVARDDLEREIKLIFAKQREYGNIVCTEAFEHEYISIWASQRP
FASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFTVWEHINKLRLVSPGGIRALTDDER
RLIYKQAPHKNKITFHDVRTLLNLPDDTRFKGLLYDRNTTLKENEKVRFLELGAYHK
IRKAIDSVYGKGAAKSFRPIDFDTFGYALTMFKDDTDIRSYLRNEYEQNGKRMENLA
DKVYDEELIEELLNLSFSKFGHLSLKALRNILPYMEQGEVYSTACERAGYTFTGPKKK
QKTVLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARELSQSFDERRKM
QKEQEGNRKKNETAIRQLVEYGLTLNPTGLDIVKFKLWSEQNGKCAYSLQPIEIERLL
EPGYTEVDHVIPYSRSLDDSYTNKVLVLTKENREKGNRTPAEYLGLGSERWQQFETF
VLTNKQFSKKKRDRLLRLHYDENEENEFKNRNLNDTRYISRFLANFIREHLKFADSD
DKQKVYTVNGRITAHLRSRWNFNKNREESNLHHAVDAAIVACTTPSDIARVTAFYQ
RREQNKELSKKTDPQFPQPWPHFADELQARLSKNPKESIKALNLGNYDNEKLESLQP
VFVSRMPKRSITGAAHQETLRRYIGIDERSGKIQTVVKKKLSEIQLDKTGHFPMYGKE
SDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGELGPIIRTIKIIDTTNQVIPLNDGK
TVAYNSNIVRVDVFEKDGKYYCVPIYTIDMMKGILPNKAIEPNKPYSEWKEMTEDYT
FRFSLYPNDLIRIEFPREKTIKTAVGEEIKIKDLFAYYQTIDSSNGGLSLVSHDNNFSLR
SIGSRTLKRFEKYQVDVLGNIYKVRGEKRVGVASSSHSKAGETIRPL
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B. GeoCas9 with Altered PAM Specificities

[0086] The GeoCas9 base editors may also comprise GeoCas9 proteins with altered PAM specificities.

[0087] Some aspects of the disclosure provide GeoCas9 proteins that have Protospacer Adjacent Motif (PAM) specificities that differ from other GeoCas9 proteins. A PAM sequence is typically a 2-6 base pair DNA sequence that immediately follows the DNA sequence targeted by a Cas9 nuclease in the CRISPR bacterial adaptive immune system. Typically Cas9 will not bind to or cleave a target DNA sequence if it is not followed by the PAM sequence. Thus, PAM requirements may limit the ability to edit desired bases within a genome. For example spCas9 proteins may be limited to binding to locations of a nucleic acid molecule having an adjacent 5'-NGG-3' sequence. In some embodiments, the base editing fusion proteins provided herein may need to be placed at a precise location, for example where a target base is placed within a 4 base region (e.g., a "deamination window"), which is approximately 15 bases upstream of the PAM. See Komor, A. C., et al., "Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage" Nature 533, 420-424 (2016), the entire contents of which are hereby incorporated by reference. In some embodiments, a target base is placed within a base region from 1 to 10 nucleotides in length. In some embodiments, a target base is placed within a base region that is at least 1, at lest 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 nucleotides in length. In some embodiments, the target base is from 5 to 25 bases upstream of the PAM. In some embodiments, the target base is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 bases upstream of the PAM.

[0088] In some embodiments, any of the fusion proteins provided herein may contain a GeoCas9 that is capable of binding a nucleotide sequence having the PAM sequence 5'-NNNNCRAA-3' (SEQ ID NO: 79), where N is A, T, C, or G, and R is A or G as indicated by the IUPAC nucleotide code naming system. In some embodiments, the GeoCas9 is capable of binding a nucleotide sequence having the PAM sequence 5'-NIN2N3N4CRAA-3', where N1, N2, N3, and/or N4 is A. In some embodiments, the GeoCas9 is capable

of binding a nucleotide sequence having the PAM sequence 5'-$N_1N_2N_3N_4$CRAA-3', where N_1, N_2, N_3, and/or N_4 is T. In some embodiments, the GeoCas9 is capable of binding a nucleotide sequence having the PAM sequence 5'-$N_1N_2N_3N_4$CRAA-3', where N_1, N_2, N_3, and/or N_4 is C. In some embodiments, the GeoCas9 is capable of binding a nucleotide sequence having the PAM sequence 5'-$N_1N_2N_3N_4$CRAA-3', where N_1, N_2, N_3, and/or N_4 is G. It should be appreciated that $N_1N_2N_3N_4$ may be a four nucleotide sequence having any combination of neucleotides selected from the group consisting of A, T, C, and G. GeoCas9 proteins that bind to 5'-NNNNCRAA-3' (SEQ ID NO: 79) PAM sequences have been described in Harrington L. B., Paez-Espino D., Staahl B. T., Chen J. C., Ma E., Kyrpides N.C., Doudna J. A. Nature Communications 8, 1424 (2017)); the entire contents of each are hereby incorporated by reference.

[0089] In some embodiments, the GeoCas9 or variant thereof is a GeoCas9 or variant thereof from the genus *Geobacillus*. In some embodiments, the GeoCas9 or variant thereof is from a species selected from the group consisting of *G. stearothermophilus, G. thermodenitrificans, G. caldoxylosilyticus, G. galactosidasius, G. icigianus, G. jurassicus, G. kaustophilus, G. lituanicus, G. subterraneus, G. thermantarcticus, G. thermocatenulatus, G. thermoglucosidasius, G. thermoleovorans, G. toebii, G. uzenensis*, and *G. vulcani*. In some embodiments, the GeoCas9 of any of the fusion proteins provided herein comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 of any of the fusion proteins provided herein comprises the amino acid sequence of any one of SEQ ID NOs: 2, 3, or 5-8. In some embodiments, the GeoCas9 protein of any of the fusion proteins provided herein consists of the amino acid sequence of any one of SEQ ID NOs: 2, 3, or 5-8.

[0090] Additional suitable GeoCas9 proteins, variants, and sequences will also be apparent to those of skill in the art. Examples of such additional suitable GeoCas9 proteins include, but are not limited to, D8A, D8A/D581A/H582A, and D8A/D581A/H582A/N605A mutant domains (see, e.g., Prashant et al., CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nature Biotechnology. 2013; 31 (9): 833-838 the entire contents of which are incorporated herein by reference). In some embodiments, the GeoCas9 comprises a histidine residue at position 582 of the amino acid sequence provided in SEQ ID NO: 3, or a corresponding mutation in any of the amino acid sequences provided in SEQ ID NOs: 2, 3, 5-8.

C. Nucleic Acid Effector Domain

[0091] The GeoCas9 base editor fusion proteins may comprise one or more nucleic acid effector domains. Exemplary nucleic acid effector domains include, but are not limited to a deaminase (e.g., a cytidine deaminase or an adenosine deaminase), a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain. In some embodiments the nucleic acid effector domain is a protein or enzyme capable of making one or more modifications (e.g., deamination of a cytidine residue) to a nucleic acid (e.g., DNA or RNA). In some embodiments the nucleic acid editing domain is a deaminase (e.g., a cytidine deaminase, such as an APOBEC or an AID deaminase). Additional suitable nucleic-acid effector enzyme sequences that can be used according to aspects of this invention, e.g., that can be fused to a GeoCas9 protein, will be apparent to those of skill in the art based on this disclosure. In some embodiments, such additional enzyme sequences include deaminase enzyme or deaminase domain sequences that are at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% similar to the sequences provided herein.

[0092] In some embodiments, the nucleic acid effector domain is a deaminase domain. In some embodiments, the deaminase is a cytosine deaminase or a cytidine deaminase. In some embodiments, the deaminase is an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the deaminase is an APOBEC1 deaminase. In some embodiments, the deaminase is an APOBEC2 deaminase. In some embodiments, the deaminase is an APOBEC3 deaminase. In some embodiments, the deaminase is an APOBEC3A deaminase. In some embodiments, the deaminase is an APOBEC3B deaminase. In some embodiments, the deaminase is an APOBEC3C deaminase. In some embodiments, the deaminase is an APOBEC3D deaminase. In some embodiments, the deaminase is an APOBEC3E deaminase. In some embodiments, the deaminase is an APOBEC3F deaminase. In some embodiments, the deaminase is an APOBEC3G deaminase. In some embodiments, the deaminase is an APOBEC3H deaminase. In some embodiments, the deaminase is an APOBEC4 deaminase. In some embodiments, the deaminase is an activation-induced deaminase (AID). In some embodiments, the demianse is a rat APOBEC1 (SEQ ID NO: 43). In some embodiments, the deminase is a human APOBEC1 (SEQ ID NO: 42). In some embodiments, the deaminase is a *Petromyzon marinus* cytidine deaminase 1 (pmCDA1) (SEQ ID NO: 53). In some embodiments, the deminase is a human APOBEC3G (SEQ ID NO: 34). In some embodiments, the deaminase is a fragment of the human APOBEC3G (SEQ ID NO: 58). In some embodiments, the deaminase is a human APOBEC3G variant comprising a D316R_D317R mutation (SEQ ID NO: 57). In some embodiments, the deaminase is a frantment of the human APOBEC3G and comprising mutations corresponding to the D316R_D317R mutations in SEQ ID NO: 34 (SEQ ID NO: 59).

[0093] One exemplary suitable type of nucleic acid effector domain is a cytidine deaminase, for example, of the APOBEC family. The apolipoprotein B mRNA-editing complex (APOBEC) family of cytidine deaminase enzymes encompasses eleven proteins that serve to initiate mutagenesis in a controlled and beneficial manner.[29] One family member, activation-induced cytidine deaminase (AID), is responsible for the maturation of antibodies by converting cytosines in ssDNA to uracils in a transcription-dependent, strand-biased fashion.[30] The apolipoprotein B editing complex 3 (APOBEC3) enzyme provides protection to human cells against a certain HIV-1 strain via the deamination of cytosines in reverse-transcribed viral ssDNA.31 These proteins all require a Zn^{2+}-coordinating motif (His-X-Glu-X_{23-26}-Pro-Cys-X_{2-4}-Cys; SEQ ID NO: 383) and bound water molecule for catalytic activity. The Glu residue acts to activate the water molecule to a zinc hydroxide for nucleo-

philic attack in the deamination reaction. Each family member preferentially deaminates at its own particular "hotspot", ranging from WRC (W is A or T, R is A or G) for hAID, to TTC for hAPOBEC3F.[32] A recent crystal structure of the catalytic domain of APOBEC3G revealed a secondary structure comprised of a five-stranded β-sheet core flanked by six α-helices, which is believed to be conserved across the entire family.[33] The active center loops have been shown to be responsible for both ssDNA binding and in determining "hotspot" identity.[34] Overexpression of these enzymes has been linked to genomic instability and cancer, thus highlighting the importance of sequence-specific targeting.[35]

[0094] Some aspects of this disclosure relate to the recognition that the activity of cytidine deaminase enzymes such as APOBEC enzymes can be directed to a specific site in genomic DNA. Without wishing to be bound by any particular theory, advantages of using GeoCas9 as a recognition agent include (1) the sequence specificity of GeoCas9 can be easily altered by simply changing the sgRNA sequence; and (2) GeoCas9 binds to its target sequence by denaturing the dsDNA, resulting in a stretch of DNA that is single-stranded and therefore a viable substrate for the deaminase; (3) GeoCas9 is a thermostable hololog of other Cas9 proteins; (4) GeoCas9 is smaller than many homologs of Cas9 proteins from other organisms; and (5) GeoCas9 recognizes and can bind to nucleic acid sequences comprising the PAM sequence 5'-NNNNCRAA-3' (SEQ ID NO: 79). It should be understood that other catalytic domains, or catalytic domains from other deaminases, can also be used to generate GeoCas9 base editor fusion proteins with GeoCas9, and that the disclosure is not limited in this regard.

[0095] Some aspects of this disclosure are based on the recognition that Cas9: deaminase fusion proteins can efficiently deaminate nucleotides at positions-15 to 15 according to the numbering scheme in FIG. **3**. In view of the results provided herein regarding the nucleotides that can be targeted by Cas9: deaminase fusion proteins, a person of skill in the art will be able to design suitable guide RNAs to target the GeoCas9 base editor fusion proteins to a target sequence that comprises a nucleotide to be deaminated. In some embodiments, the deamination window is from position −15 to 15. In some embodiments, the deamination window is from position −15 to 10, from −15 to 5, from −15 to 0, from −15 to −5, from −15 to −10, from −10 to 15, from −10 to 10, from −10 to 5, from −10 to 0, from −10 to −5, from −5 to 15, from −5 to 10, from −5 to 5, from −5 to 0, from 0 to 15, from 0 to 10, from 0 to 5, from 5 to 15, from 5 to 10, or from 10 to 15 according to the numbering scheme in SEQ ID NO: 406.

D. Cytidine Deaminases

[0096] In some embodiments, the GeoCas9 base editors may comprise a cytidine deaminase as a nucleic acid effector domain. In some embodiments, the deaminase is an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the deaminase is an APOBEC1 deaminase. In some embodiments, the deaminase is an APOBEC2 deaminase. In some embodiments, the deaminase is an APOBEC3 deaminase. In some embodiments, the deaminase is an APOBEC3A deaminase. In some embodiments, the deaminase is an APOBEC3B deaminase. In some embodiments, the deaminase is an APOBEC3C deaminase. In some embodiments, the deaminase is an APOBEC3D deaminase. In some embodiments, the deaminase is an APOBEC3E deaminase. In some embodiments, the deaminase is an APOBEC3F deaminase. In some embodiments, the deaminase is an APOBEC3G deaminase. In some embodiments, the deaminase is an APOBEC3H deaminase. In some embodiments, the deaminase is an APOBEC4 deaminase. In some embodiments, the deaminase is an activation-induced deaminase (AID). In some embodiments, the deaminase is a vertebrate deaminase. In some embodiments, the deaminase is an invertebrate deaminase. In some embodiments, the deaminase is a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse deaminase. In some embodiments, the deaminase is a human deaminase. In some embodiments, the deaminase is a rat deaminase, e.g., rAPOBEC1. In some embodiments, the deaminase is an activation-induced cytidine deaminase (AID). In some embodiments, the deaminase is a cytidine deaminase 1 (CDA1). In some embodiments, the deaminase is a *Petromyzon marinus* cytidine deaminase 1 (pmCDA1). In some embodiments, the deminase is a human APOBEC3G (SEQ ID NO: 34). In some embodiments, the deaminase is a fragment of the human APOBEC3G (SEQ ID NO: 58). In some embodiments, the deaminase is a human APOBEC3G variant comprising a D316R_D317R mutation (SEQ ID NO: 57). In some embodiments, the deaminase is a frantment of the human APOBEC3G and comprising mutations corresponding to the D316R_D317R mutations in SEQ ID NO: 34 (SEQ ID NO: 59).

[0097] In some embodiments, the nucleic acid editing domain is at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the deaminase domain of any one of SEQ ID NOs: 25-59. In some embodiments, the nucleic acid editing domain comprises the amino acid sequence of any one of SEQ ID NOs: 25-59.

[0098] A cytidine deaminase domain may also be referred to interchangeably as a cytosine deaminase domain. In some embodiments, the cytidine deaminase catalyzes the hydrolytic deamination of cytidine (C) or deoxycytidine (dC) to uridine (U) or deoxyuridine (dU), respectively. In some embodiments, the cytidine deaminase domain catalyzes the hydrolytic deamination of cytosine (C) to uracil (U). In some embodiments, the cytidine deaminase catalyzes the hydrolytic deamination of cytidine or cytosine in deoxyribonucleic acid (DNA). Without wishing to be bound by any particular theory, fusion proteins comprising a cytidine deaminase are useful inter alia for targeted editing, referred to herein as "base editing," of nucleic acid sequences in vitro and in vivo.

[0099] One exemplary suitable type of cytidine deaminase is a cytidine deaminase, for example, of the APOBEC family. The apolipoprotein B mRNA-editing complex (APOBEC) family of cytidine deaminase enzymes encompasses eleven proteins that serve to initiate mutagenesis in a controlled and beneficial manner (see, e.g., Conticello S G. The AID/APOBEC family of nucleic acid mutators. Genome Biol. 2008; 9 (6): 229). One family member, activation-induced cytidine deaminase (AID), is responsible for the maturation of antibodies by converting cytosines in ssDNA to uracils in a transcription-dependent, strand-biased fashion (see, e.g., Reynaud C A, et al. What role for AID: mutator, or assembler of the immunoglobulin mutasome, Nat Immunol. 2003; 4 (7): 631-638). The apolipoprotein B editing complex 3 (APOBEC3) enzyme provides protection to human cells against a certain HIV-1 strain via the deami-

nation of cytosines in reverse-transcribed viral ssDNA (see, e.g., Bhagwat A S. DNA-cytosine deaminases: from antibody maturation to antiviral defense. DNA Repair (Amst). 2004; 3 (1): 85-89). These proteins all require a Zn2+-coordinating motif (His-X-Glu-X23-26-Pro-Cys-X2-4-Cys; SEQ ID NO: 383) and bound water molecule for catalytic activity. The Glu residue acts to activate the water molecule to a zinc hydroxide for nucleophilic attack in the deamination reaction. Each family member preferentially deaminates at its own particular "hotspot", ranging from WRC (W is A or T, R is A or G) for hAID, to TTC for hAPOBEC3F (see, e.g., Navaratnam N and Sarwar R. An overview of cytidine deaminases. Int J Hematol. 2006; 83 (3): 195-200). A recent crystal structure of the catalytic domain of APOBEC3G revealed a secondary structure comprised of a five-stranded β-sheet core flanked by six α-helices, which is believed to be conserved across the entire family (see, e.g., Holden L G, et al. Crystal structure of the anti-viral APOBEC3G catalytic domain and functional implications. Nature. 2008; 456 (7218): 121-4). The active center loops have been shown to be responsible for both ssDNA binding and in determining "hotspot" identity (see, e.g., Chelico L, et al. Biochemical basis of immunological and retroviral responses to DNA-targeted cytosine deamination by activation-induced cytidine deaminase and APOBEC3G. J Biol Chem. 2009; 284 (41). 27761-5). Overexpression of these enzymes has been linked to genomic instability and cancer, thus highlighting the importance of sequence-specific targeting (see, e.g., Pham P, et al. Reward versus risk: DNA cytidine deaminases triggering immunity and disease. Biochemistry. 2005; 44 (8): 2703-15).

[0100] Some aspects of this disclosure relate to the recognition that the activity of cytidine deaminase enzymes such as APOBEC enzymes can be directed to a specific site in genomic DNA. Without wishing to be bound by any particular theory, advantages of using a nucleic acid programmable binding protein (e.g., a Cas9 domain) as a recognition agent include (1) the sequence specificity of nucleic acid programmable binding protein (e.g., a Cas9 domain) can be easily altered by simply changing the sgRNA sequence; and (2) the nucleic acid programmable binding protein (e.g., a Cas9 domain) may bind to its target sequence by denaturing the dsDNA, resulting in a stretch of DNA that is single-stranded and therefore a viable substrate for the deaminase. It should be understood that other catalytic domains of napDNAbps, or catalytic domains from other nucleic acid editing proteins, can also be used to generate fusion proteins with Cas9, and that the disclosure is not limited in this regard.

[0101] In view of the results provided herein regarding the nucleotides that can be targeted by Cas9: deaminase fusion proteins, a person of ordinary skill in the art will be able to design suitable guide RNAs to target the fusion proteins to a target sequence that comprises a nucleotide to be deaminated.

[0102] In some embodiments, the cytidine deaminase is an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the cytidine deaminase is an APOBEC1 deaminase. In some embodiments, the cytidine deaminase is an APOBEC2 deaminase. In some embodiments, the cytidine deaminase is an APOBEC3 deaminase. In some embodiments, the cytidine deaminase is an APOBEC3A deaminase. In some embodiments, the cytidine deaminase is an APOBEC3B deaminase. In some embodiments, the cytidine deaminase is an APOBEC3C deaminase. In some embodiments, the cytidine deaminase is an APOBEC3D deaminase. In some embodiments, the cytidine deaminase is an APOBEC3E deaminase. In some embodiments, the cytidine deaminase is an APOBEC3F deaminase. In some embodiments, the cytidine deaminase is an APOBEC3G deaminase. In some embodiments, the cytidine deaminase is an APOBEC3H deaminase. In some embodiments, the cytidine deaminase is an APOBEC4 deaminase. In some embodiments, the cytidine deaminase is an activation-induced deaminase (AID). In some embodiments, the cytidine deaminase is a vertebrate cytidine deaminase. In some embodiments, the cytidine deaminase is an invertebrate cytidine deaminase. In some embodiments, the cytidine deaminase is a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse deaminase. In some embodiments, the cytidine deaminase is a human cytidine deaminase. In some embodiments, the cytidine deaminase is a rat cytidine deaminase, e.g., rAPOBEC1. In some embodiments, the cytidine deaminase is a *Petromyzon marinus* cytidine deaminase 1 (pmCDA1) (SEQ ID NO: 56). In some embodiments, the cytidine deaminase is a human APOBEC3G (SEQ ID NO: 34). In some embodiments, the cytidine deaminase is a fragment of the human APOBEC3G. In some embodiments, the deaminase is a human APOBEC3G variant comprising a D316R and D317R mutation. In some embodiments, the deaminase is a fragment of the human APOBEC3G and comprising mutations corresponding to the D316R and D317R mutations in SEQ ID NO: 59.

[0103] In some embodiments, the nucleic acid editing domain is at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the cytidine deaminase domain of any one of SEQ ID NOs: 25-59. In some embodiments, the nucleic acid editing domain comprises the amino acid sequence of any one of SEQ ID NOs: 25-59.

[0104] Some exemplary suitable nucleic-acid editing domains, e.g., cytidine deaminases and cytidine deaminase domains, that can be fused to a GeoCas9 domain according to aspects of this disclosure are provided below. It should be understood that, in some embodiments, the active domain of the respective sequence can be used, e.g., the domain without a localizing signal (nuclear localization sequence, without nuclear export signal, cytoplasmic localizing signal). The following are exemplary cytidine deaminases that may be used in the GeoCas9 base editor fusion protein disclosed herein.

Human AID:
(SEQ ID NO: 25)
MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYL

RNKNGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLS

LRIFTARLYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAW

EGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence;
double underline: nuclear export signal)

Mouse AID:
(SEQ ID NO: 26)
MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGH

LRNKSGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNL

SLRIFTARLYFCEDRKAEPEGLRRLHRAGVQIGIMTFKDYFYCWNTFVENRERTFKA

WEGLHENSVRLTRQLRRILLPLYEVDDLRDAFRMLGF
(underline: nuclear localization sequence;
double underline: nuclear export signal)

Dog AID:
(SEQ ID NO: 27)
MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHL

RNKSGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLS

LRIFAARLYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENREKTFKAW

EGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence;
double underline: nuclear export signal)

Bovine AID:
(SEQ ID NO: 28)
MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHL

RNKAGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLS

LRIFTARLYFCDKERKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKA

WEGLHENSVRLSRQLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence;
double underline: nuclear export signal)

Rat AID:
(SEQ ID NO: 44)
MAVGSKPKAALVGPHWERERIWCFLCSTGLGTQQTGQTSRWLRPAATQD

PVSPPRSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGYLRNK

SGCHVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIF

TARLTGWGALPAGLMSPARPSDYFYCWNTFVENHERTFKAWEGLHENSVRLSRRL

RRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence;
double underline: nuclear export signal)

Mouse APOBEC-3:
(SEQ ID NO: 29)
MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLGYAKGRKDTFLCYEVTRK

DCDSPVSLHHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFEC

AEQIVRFLATHHNLSLDIFSSRLYNVQDPETQQNLCRLVQEGAQVAAMDLYEFKKC

WKKFVDNGGRRFRPWKRLLTNFRYQDSKLQEILRPCYIPVPSSSSSTLSNICLTKGLP

-continued

ETRFCVEGRRMDPLSEEEFYSQFYNQRVKHLCYYHRMKPYLCYQLEQFNGQAPLKG

CLLSEKGKQHAEILFLDKIRSMELSQVTITCYLTWSPCPNCAWQLAAFKRDRPDLILH

IYTSRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGL

EIISRRTQRRLRRIKESWGLQDLVNDFGNLQLGPPMS
(italic: nucleic acid editing domain)

Rat APOBEC-3:
(SEQ ID NO: 30)
MGPFCLGCSHRKCYSPIRNLISQETFKFHFKNLRYAIDRKDTFLCYEVTRKD

CDSPVSLHHGVFKNKDNIHAEICFLYWFHDKVLKVLSPREEFKITWYMSWSPCFECA

EQVLRFLATHHNLSLDIFSSRLYNIRDPENQQNLCRLVQEGAQVAAMDLYEFKKCW

KKFVDNGGRRFRPWKKLLTNFRYQDSKLQEILRPCYIPVPSSSSSTLSNICLTKGLPET

RFCVERRRVHLLSEEEFYSQFYNQRVKHLCYYHGVKPYLCYQLEQFNGQAPLKGCL

LSEKGKQHAEILFLDKIRSMELSQVIITCYLTWSPCPNCAWQLAAFKRDRPDLILHIYT

SRLYFHWKRPFQKGLCSLWQSGILVDVMDLPQFTDCWTNFVNPKRPFWPWKGLEII

SRRTQRRLHRIKESWGLQDLVNDFGNLQLGPPMS
(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3G:
(SEQ ID NO: 31)
MVEPMDPRTFVSNFNNRPILSGLNTVWLCCEVKTKDPSGPPLDAKIFQGKV

YSKAKYHPEMRFLRWFHKWRQLHHDQEYKVTWYVSWSPCTRCANSVATFLAKDP

KVTLTIFVARLYYFWKPDYQQALRILCQKRGGPHATMKIMNYNEFQDCWNKFVDG

RGKPFKPRNNLPKHYTLLQATLGELLRHLMDPGTFTSNFNNKPWVSGQHETYLCYK

VERLHNDTWVPLNQHRGFLRNQAPNIHGFPKGRHAELCFLDLIPFWKLDGQQYRVT

CFTSWSPCFSCAQEMAKFISNNEHVSLCIFAARIYDDQGRYQEGLRALHRDGAKIAM

MNYSEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI
(italic: nucleic acid editing domain;
underline: cytoplasmic localization signal)

Chimpanzee APOBEC-3G:
(SEQ ID NO: 32)
MKPHFRNPVERMYQDTFSDNFYNRPILSHRNTVWLCYEVKTKGPSRPPLD

AKIFRGQVYSKLKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDVA

TFLAEDPKVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCW

SKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTSNFNNELWVRGRHET

YLCYEVERLHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLH

QDYRVTCFTSWSPCFSCAQEMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLAKA

GAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLEEHSQALSGRLRAILQNQGN
(italic: nucleic acid editing domain;
underline: cytoplasmic localization signal)

Green monkey APOBEC-3G:
(SEQ ID NO: 33)
MNPQIRNMVEQMEPDIFVYYFNNRPILSGRNTVWLCYEVKTKDPSGPPLD

ANIFQGKLYPEAKDHPEMKFLHWFRKWRQLHRDQEYEVTWYVSWSPCTRCANSVA

TFLAEDPKVTLTIFVARLYYFWKPDYQQALRILCQERGGPHATMKIMNYNEFQHCW

NEFVDGQGKPFKPRKNLPKHYTLLHATLGELLRHVMDPGTFTSNFNNKPWVSGQRE

-continued

TYLCYKVERSHNDTWVLLNQHRGFLRNQAPDRHGFPKGRHAELCFLDLIPFWKLDD

QQYRVTCFTSWSPCFSCAQKMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLHRD

GAKIAVMNYSEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI
(italic: nucleic acid editing domain;
underline: cytoplasmic localization signal)

Human APOBEC-3G:
(SEQ ID NO: 34)
MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLD

AKIFRGQVYSELKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMA

TFLAEDPKVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCW

SKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETY

LCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLD

QDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEA

GAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN
(italic: nucleic acid editing domain;
underline: cytoplasmic localization signal)

Human APOBEC-3F:
(SEQ ID NO: 35)
MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLD

AKIFRGQVYSQPEHHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLAEF

LAEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYS

EGQPFMPWYKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWL

CFTMEVVKHHSPVSWKRGVFRNQVDPETHCHAERCFLSWFCDDILSPNTNYEVTWY

TSWSPCPECAGEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIM

GYKDFKYCWENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE
(italic: nucleic acid editing domain)

Human APOBEC-3B:
(SEQ ID NO: 36)
MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLW

DTGVFRGQVYFKPQYHAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDCVAKLA

EFLSEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVTIMDYEEFAYCWENFV

YNEGQQFMPWYKFDENYAFLHRTLKEILRYLMDPDTFTFNFNNDPLVLRRRQTYLC

YEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFYGRHAELRFLDLVPSLQLDPAQI

YRVTWFISWSPCFSWGCAGEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRD

AGAQVSIMTYDEFEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQNQGN
(italic: nucleic acid editing domain)

Rat APOBEC-3B:
(SEQ ID NO: 48)
MQPQGLGPNAGMGPVCLGCSHRRPYSPIRNPLKKLYQQTFYFHFKNVRYA

WGRKNNFLCYEVNGMDCALPVPLRQGVFRKQGHIHAELCFIYWFHDKVLRVLSPM

EEFKVTWYMSWSPCSKCAEQVARFLAAHRNLSLAIFSSRLYYYLRNPNYQQKLCRLI

QEGVHVAAMDLPEFKKCWNKFVDNDGQPFRPWMRLRINFSFYDCKLQEIFSRMNLL

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REDVFYLQFNNSHRVKPVQNRYYRRKSYLCYQLERANGQEPLKGYLLYKKGEQHV

EILFLEKMRSMELSQVRITCYLTWSPCPNCARQLAAFKKDHPDLILRIYTSRLYFYWR

KKFQKGLCTLWRSGIHVDVMDLPQFADCWTNFVNPQRPFRPWNELEKNSWRIQRR

LRRIKESWGL

Bovine APOBEC-3B:
(SEQ ID NO: 49)
DGWEVAFRSGTVLKAGVLGVSMTEGWAGSGHPGQGACVWTPGTRNTM

NLLREVLFKQQFGNQPRVPAPYYRRKTYLCYQLKQRNDLTLDRGCFRNKKQRHAEI

RFIDKINSLDLNPSQSYKIICYITWSPCPNCANELVNFITRNNHLKLEIFASRLYFHWIK

SFKMGLQDLQNAGISVAVMTHTEFEDCWEQFVDNQSRPFQPWDKLEQYSASIRRRL

QRILTAPI

Chimpanzee APOBEC-3B:
(SEQ ID NO: 50)
MNPQIRNPMEWMYQRTFYYNFENEPILYGRSYTWLCYEVKIRRGHSNLL

WDTGVFRGQMYSQPEHHAEMCFLSWFCGNQLSAYKCFQITWFVSWTPCPDCVAKL

AKFLAEHPNVTLTISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENF

VYNEGQPFMPWYKFDDNYAFLHRTLKEIIRHLMDPDTFTFNFNNDPLVLRRHQTYL

CYEVERLDNGTWVLMDQHMGFLCNEAKNLLCGFYGRHAELRFLDLVPSLQLDPAQI

YRVTWFISWSPCFSWGCAGQVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRD

AGAQVSIMTYDEFEYCWDTFVYRQGCPFQPWDGLEEHSQALSGRLRAILQVRASSL

CMVPHRPPPPPQSPGPCLPLCSEPPLGSLLPTGRPAPSLPFLLTASFSFPPPASLPPLPSL

SLSPGHLPVPSFHSLTSCSIQPPCSSRIRETEGWASVSKEGRDLG

Human APOBEC-3C:
(SEQ ID NO: 37)
MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVS

WKTGVFRNQVDSETHCHAERCFLSWFCDDILSPNTKYQVTWYTSWSPCPDCAGEV

AEFLARHSNVNLTIFTARLYYFQYPCYQEGLRSLSQEGVAVEIMDYEDFKYCWENFV

YNDNEPFKPWKGLKTNFRLLKRRLRESLQ
(italic: nucleic acid editing domain)

Gorilla APOBEC3C:
(SEQ ID NO: 45)
MNPQIRNPMKAMYPGTFYFQFKNLWEANDRNETWLCFTVEGIKRRSVVSWKTGVF

RNQVDSETHCHAERCFLSWFCDDILSPNTNYQVTWYTSWSPCPECAGEVAEFLARH

SNVNLTIFTARLYYFQDTDYQEGLRSLSQEGVAVKIMDYKDFKYCWENFVYNDDEP

FKPWKGLKYNFRFLKRRLQEILE
(italic: nucleic acid editing domain)

Human APOBEC-3A:
(SEQ ID NO: 38)
MEASPASGPRHLMDPHIFTSNFNNGIGRHKTYLCYEVERLDNGTSVKMDQ

HRGFLHNQAKNLLCGFYGRHAELRFLDLVPSLQLDPAQIYRVTWFISWSPCFSWGCA

GEVRAFLQENTHVRLRIFAARIYDYDPLYKEALQMLRDAGAQVSIMTYDEFKHCWD

TFVDHQGCPFQPWDGLDEHSQALSGRLRAILQNQGN
(italic: nucleic acid editing domain)

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Rhesus macaque APOBEC-3A:
(SEQ ID NO: 46)
MDGSPASRPRHLMDPNTFTFNFNNDLSVRGRHQTYLCYEVERLDNGTWVPMDERR

GFLCNKAKNVPCGDYGCHVELRFLCEVPSWQLDPAQTYRVTWFISWSPCFRRGCAG

QVRVFLQENKHVRLRIFAARIYDYDPLYQEALRTLRDAGAQVSIMTYEEFKHCWDT

FVDRQGRPFQPWDGLDEHSQALSGRLRAILQNQGN
(italic: nucleic acid editing domain)

Bovine APOBEC-3A:
(SEQ ID NO: 47)
MDEYTFTENFNNQGWPSKTYLCYEMERLDGDATIPLDEYKGFVRNKGLDQPEKPCH

AELYFLGKIHSWNLDRNQHYRLTCFISWSPCYDCAQKLTTFLKENHHISLHILASRIY

THNRFGCHQSGLCELQAAGARITIMTFEDFKHCWETFVDHKGKPFQPWEGLNVKSQ

ALCTELQAILKTQQN
(italic: nucleic acid editing domain)

Human APOBEC-3H:
(SEQ ID NO: 39)
MALLTAETFRLQFNNKRRLRRPYYPRKALLCYQLTPQNGSTPTRGYFENK

KKCHAEICFINEIKSMGLDETQCYQVTCYLTWSPCSSCAWELVDFIKAHDHLNLGIFA

SRLYYHWCKPQQKGLRLLCGSQVPVEVMGFPKFADCWENFVDHEKPLSFNPYKML

EELDKNSRAIKRRLERIKIPGVRAQGRYMDILCDAEV
(italic: nucleic acid editing domain)

Rhesus macaque APOBEC-3H:
(SEQ ID NO: 51)
MALLTAKTFSLQFNNKRRVNKPYYPRKALLCYQLTPQNGSTPTRGHLKNK

KKDHAEIRFINKIKSMGLDETQCYQVTCYLTWSPCPSCAGELVDFIKAHRHLNLRIFA

SRLYYHWRPNYQEGLLLLCGSQVPVEVMGLPEFTDCWENFVDHKEPPSFNPSEKLE

ELDKNSQAIKRRLERIKSRSVDVLENGLRSLQLGPVTPSSSIRNSR

Human APOBEC-3D:
(SEQ ID NO: 40)
MNPQIRNPMERMYRDTFYDNFENEPILYGRSYTWLCYEVKIKRGRSNLLW

DTGVFRGPVLPKRQSNHRQEVYFRFENHAEMCFLSWFCGNRLPANRRFQITWFVSW

NPCLPCVVKVTKFLAEHPNVTLTISAARLYYYRDRDWRWVLLRLHKAGARVKIMD

YEDFAYCWENFVCNEGQPFMPWYKFDDNYASLHRTLKEILRNPMEAMYPHIFYFHF

KNLLKACGRNESWLCFTMEVTKHHSAVFRKRGVFRNQVDPETHCHAERCFLSWFC

DDILSPNTNYEVTWYTSWSPCPECAGEVAEFLARHSNVNLTIFTARLCYFWDTDYQE

GLCSLSQEGASVKIMGYKDFVSCWKNFVYSDDEPFKPWKGLQTNFRLLKRRLREILQ
(italic: nucleic acid editing domain)

Human APOBEC-1:
(SEQ ID NO: 41)
MTSEKGPSTGDPTLRRRIEPWEFDVFYDPRELRKEACLLYEIKWGMSRKIW

RSSGKNTTNHVEVNFIKKFTSERDFHPSMSCSITWFLSWSPCWECSQAIREFLSRHPG

VTLVIYVARLFWHMDQQNRQGLRDLVNSGVTIQIMRASEYYHCWRNFVNYPPGDE

AHWPQYPPLWMMLYALELHCIILSLPPCLKISRRWQNHLTFFRLHLQNCHYQTIPPHI

LLATGLIHPSVAWR

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Mouse APOBEC-1:
(SEQ ID NO: 42)
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSVW

RHTSQNTSNHVEVNFLEKFTTERYFRPNTRCSITWFLSWSPCGECSRAITEFLSRHPY

VTLFIYIARLYHHTDQRNRQGLRDLISSGVTIQIMTEQEYCYCWRNFVNYPPSNEAY

WPRYPHLWVKLYVLELYCIILGLPPCLKILRRKQPQLTFFTITLQTCHYQRIPPHLLWA

TGLK

RatAPOBEC-1:
(SEQ ID NO: 43)
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWR

HTSQNTNKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVT

LFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPR

YPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGLK

Human APOBEC-2:
(SEQ ID NO: 52)
MAQKEEAAVATEAASQNGEDLENLDDPEKLKELIELPPFEIVTGERLPANF

FKFQFRNVEYSSGRNKTFLCYVVEAQGKGGQVQASRGYLEDEHAAAHAEEAFFNTI

LPAFDPALRYNVTWYVSSSPCAACADRIIKTLSKTKNLRLLILVGRLFMWEEPEIQAA

LKKLKEAGCKLRIMKPQDFEYVWQNFVEQEEGESKAFQPWEDIQENFLYYEEKLAD

ILK

Mouse APOBEC-2:
(SEQ ID NO: 53)
MAQKEEAAEAAAPASQNGDDLENLEDPEKLKELIDLPPFEIVTGVRLPVNF

FKFQFRNVEYSSGRNKTFLCYVVEVQSKGGQAQATQGYLEDEHAGAHAEEAFFNTI

LPAFDPALKYNVTWYVSSSPCAACADRILKTLSKTKNLRLLILVSRLFMWEEPEVQA

ALKKLKEAGCKLRIMKPQDFEYIWQNFVEQEEGESKAFEPWEDIQENFLYYEEKLAD

ILK

Rat APOBEC-2:
(SEQ ID NO: 54)
MAQKEEAAEAAAPASQNGDDLENLEDPEKLKELIDLPPFEIVTGVRLPVNF

FKFQFRNVEYSSGRNKTFLCYVVEAQSKGGQVQATQGYLEDEHAGAHAEEAFFNTI

LPAFDPALKYNVTWYVSSSPCAACADRILKTLSKTKNLRLLILVSRLFMWEEPEVQA

ALKKLKEAGCKLRIMKPQDFEYLWQNFVEQEEGESKAFEPWEDIQENFLYYEEKLA

DILK

Bovine APOBEC-2:
(SEQ ID NO: 55)
MAQKEEAAAAAEPASQNGEEVENLEDPEKLKELIELPPFEIVTGERLPAHYFKFQFRN

VEYSSGRNKTFLCYVVEAQSKGGQVQASRGYLEDEHATNHAEEAFFNSIMPTFDPA

LRYMVTWYVSSSPCAACADRIVKTLNKTKNLRLLILVGRLFMWEEPEIQAALRKLKE

AGCRLRIMKPQDFEYIWQNFVEQEEGESKAFEPWEDIQENFLYYEEKLADILK

Petromyzon marinus CDA1 (pmCDA1):
(SEQ ID NO: 56)
MTDAEYVRIHEKLDIYTFKKQFFNNKKSVSHRCYVLFELKRRGERRACFW

GYAVNKPQSGTERGIHAEIFSIRKVEEYLRDNPGQFTINWYSSWSPCADCAEKILEWY

NQELRGNGHTLKIWACKLYYEKNARNQIGLWNLRDNGVGLNVMVSEHYQCCRKIF

IQSSHNQLNENRWLEKTLKRAEKRRSELSIMIQVKILHTTKSPAV

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Human APOBEC3G D316R D317R:

(SEQ ID NO: 57)

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLD

AKIFRGQVYSELKYHPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKCTRDMA

TFLAEDPKVTLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCW

SKFVYSQRELFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETY

LCYEVERMHNDTWVLLNQRRGFLCNQAPHKHGFLEGRHAELCFLDVIPFWKLDLD

QDYRVTCFTSWSPCFSCAQEMAKFISKNKHVSLCIFTARIYRRQGRCQEGLRTLAEA

GAKISIMTYSEFKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN

Human APOBEC3G chain A:

(SEQ ID NO: 58)

MDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQ

APHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKN

KHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQGCPFQ

PWDGLDEHSQDLSGRLRAILQ

Human APOBEC3G chain A D120R_D121R:

(SEQ ID NO: 59)

MDPPTFTFNFNNEPWVRGRHETYLCYEVERMHNDTWVLLNQRRGFLCNQ

APHKHGFLEGRHAELCFLDVIPFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKN

KHVSLCIFTARIYRRQGRCQEGLRTLAEAGAKISIMTYSEFKHCWDTFVDHQGCPFQ

PWDGLDEHSQDLSGRLRAILQ

[0105] Some aspects of the disclosure are based on the recognition that modulating the deaminase domain catalytic activity of any of the fusion proteins provided herein, for example by making point mutations in the deaminase domain, affect the processivity of the fusion proteins (e.g., base editors). For example, mutations that reduce, but do not eliminate, the catalytic activity of a deaminase domain within a base editing fusion protein can make it less likely that the deaminase domain will catalyze the deamination of a residue adjacent to a target residue, thereby narrowing the deamination window. The ability to narrow the deamina-taion window may prevent unwanted deamination of residues adjacent of specific target residues, which may decrease or prevent off-target effects.

[0106] In some embodiments, any of the fusion proteins provided herein comprise a deaminase domain (e.g., a cytidine deaminase domain) that has reduced catalytic deaminase activity. In some embodiments, any of the fusion proteins provided herein comprise a deaminase domain (e.g., a cytidine deaminase domain) that has a reduced catalytic deaminase activity as compared to an appropriate control. For example, the appropriate control may be the deaminase activity of the deaminase prior to introducing one or more mutations into the deaminase. In other embodiments, the appropriate control may be a wild-type deaminase. In some embodiments, the appropriate control is a wild-type apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the appropriate control is an APOBEC1 deaminase, an APOBEC2 deaminase, an APOBEC3A deaminase, an APOBEC3B deaminase, an APOBEC3C deaminase, an APOBEC3D deaminase, an APOBEC3F deaminase, an APOBEC3G deaminase, or an APOBEC3H deaminase. In some embodiments, the appropriate control is an activation induced deaminase (AID). In some embodiments, the appropriate control is a cytidine deaminase 1 from *Petromyzon marinus* (pmCDA1). In some embodiments, the deaminse domain may be a deaminase domain that has at least 1%, at least 5%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at lest 70%, at least 80%, at least 90%, or at least 95% less catalytic deaminase activity as compared to an appropriate control.

[0107] In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising one or more mutations selected from the group consisting of H121X, H122X, R126X, R126X, R118X, W90X, W90X, and R132X of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase, wherein X is any amino acid. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising one or more mutations selected from the group consisting of H121R, H122R, R126A, R126E, R118A, W90A, W90Y, and R132E of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase.

[0108] In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising one or more mutations selected from the group consisting of D316X, D317X, R320X, R320X, R313X, W285X, W285X, R326X of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase, wherein X is any amino acid. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising one or more mutations selected from the group consisting of D316R, D317R, R320A, R320E, R313A, W285A, W285Y, R326E of

hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase.

[0109] In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a H121R and a H122Rmutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R126A mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R126E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R118A mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W90A mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W90Y mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R132E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W90Y and a R126E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R126E and a R132E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W90Y and a R132E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W90Y, R126E, and R132E mutation of rAPOBEC1 (SEQ ID NO: 43), or one or more corresponding mutations in another APOBEC deaminase.

[0110] In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a D316R and a D317R mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R320A mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R320E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R313A mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W285A mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W285Y mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R326E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W285Y and a R320E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a R320E and a R326E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W285Y and a R326E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase. In some embodiments, any of the fusion proteins provided herein comprise an APOBEC deaminase comprising a W285Y, R320E, and R326E mutation of hAPOBEC3G (SEQ ID NO: 34), or one or more corresponding mutations in another APOBEC deaminase.

E. Adenosine Deaminases

[0111] In still other embodiments, the GeoCas9 base editors may comprise an adenosine deaminase as the nucleic acid effector domain.

[0112] Without wishing to be bound by any particular theory, dimerization of adenosine deaminases (e.g., in cis or in trans) may improve the ability (e.g., efficiency) of the fusion protein to modify a nucleic acid base, for example to deaminate adenine. In some embodiments, any of the GeoCas9 base editors may comprise 2, 3, 4 or 5 adenosine deaminases. In some embodiments, any of the GeoCas9 base editors provided herein comprise two adenosine deaminases. Exemplary, non-limiting, embodiments of adenosine deaminases are provided herein. It should be appreciated that the mutations provided herein (e.g., mutations in ecTadA) may be applied to adenosine deaminases in other adenosine base editors, for example those provided in U.S. Patent Publication No. 2018/0073012, published Mar. 15, 2018, which issued as U.S. Pat. No. 10,113,163, on Oct. 30, 2018; U.S. Patent Publication No. 2017/0121693, published May 4, 2017, which issued as U.S. Pat. No. 10,167,457 on Jan. 1, 2019; International Publication No. WO 2017/070633, published Apr. 27, 2017; U.S. Patent Publication No. 2015/0166980, published Jun. 18, 2015; U.S. Pat. No. 9,840,699, issued Dec. 12, 2017; and U.S. Pat. No. 10,077,453, issued Sep. 18, 2018, all of which are incorporated herein by reference in their entireties.

[0113] In some embodiments, any of the adenosine deaminases provided herein is capable of deaminating adenine. In some embodiments, the adenosine deaminases provided herein are capable of deaminating adenine in a deoxyadenosine residue of DNA. The adenosine deaminase may be

derived from any suitable organism (e.g., *E. coli*). In some embodiments, the adenosine deaminase is a naturally-occurring adenosine deaminase that includes one or more mutations corresponding to any of the mutations provided herein (e.g., mutations in ecTadA). One of skill in the art will be able to identify the corresponding residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues. Accordingly, one of skill in the art would be able to generate mutations in any naturally-occurring adenosine deaminase (e.g., having homology to ecTadA) that corresponds to any of the mutations described herein, e.g., any of the mutations identified in ecTadA. In some embodiments, the adenosine deaminase is from a prokaryote. In some embodiments, the adenosine deaminase is from a bacterium. In some embodiments, the adenosine deaminase is from *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Shewanella putrefaciens, Haemophilus influenzae, Caulobacter crescentus*, or *Bacillus subtilis*. In some embodiments, the adenosine deaminase is from *E. coli.*

[0114] In some embodiments, the adenosine deaminase comprises an amino acid sequence that is at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to any one of the amino acid sequences set forth in any one of SEQ ID NOs: 413-434, or to any of the adenosine deaminases provided herein. It should be appreciated that adenosine deaminases provided herein may include one or more mutations (e.g., any of the mutations provided herein). The disclosure provides adenosine deaminases with a certain percent identity plus any of the mutations or combinations thereof described herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 21, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more mutations compared to any one of the amino acid sequences set forth in SEQ ID NOs: 413-434, or any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminase comprises an amino acid sequence that has at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, or at least 170 identical contiguous amino acid residues as compared to any one of the amino acid sequences set forth in SEQ ID NOs: 413-434, or any of the adenosine deaminases provided herein.

[0115] In some embodiments, the adenosine deaminase comprises an E59X mutation in ecTadA SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In particular embodiments, the adenosine deaminase comprises a E59A mutation in SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase.

[0116] In some embodiments, the adenosine deaminase comprises a D108X mutation in ecTadA SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a D108W, D108Q, D108F, D108K, or D108M mutation in SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase. In particular embodiments, the adenosine deaminase comprises a D108W mutation in SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase. It should be appreciated, however, that additional deaminases may similarly be aligned to identify homologous amino acid residues that may be mutated as provided herein.

[0117] In some embodiments, the adenosine deaminase comprises TadA 7.10, whose sequence is provided as SEQ ID NO: 423, or a variant thereof. TadA7.10 comprises the following mutations in ecTadA: W23R, H36L, P48A, R51L, L84F, A106V, D108N, H123Y, S146C, D147Y, R152P, E155V, 1156F, K157N.

[0118] In particular embodiments, the adenosine deaminase comprises an N108W mutation in SEQ ID NO: 423, an embodiment also referred to as TadA 7.10 (N108W). Its sequence is provided as SEQ ID NO: 425.

[0119] In some embodiments, the adenosine deaminase comprises an A106X mutation in ecTadA SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase, where X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106V mutation in SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase. In some embodiments, the adenosine deaminase comprises an A106Q, A106F, A106W, or A106M mutation in SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase.

[0120] In particular embodiments, the adenosine deaminase comprises a V106W mutation in SEQ ID NO: 423, an embodiment also referred to as TadA 7.10 (V106W). Its sequence is provided as SEQ ID NO: 424.

[0121] In some embodiments, the adenosine deaminase comprises a R47X mutation in SEQ ID NO: 425, or a corresponding mutation in another adenosine deaminase, where the presence of X indicates any amino acid other than the corresponding amino acid in the wild-type adenosine deaminase. In some embodiments, the adenosine deaminase comprises a R47Q, R47F, R47W, or R47M mutation in SEQ ID NO: 425, or a corresponding mutation in another adenosine deaminase.

[0122] In particular embodiments, the adenosine deaminase comprises a R47Q, R47F, R47W, or R47M mutation in SEQ ID NO: 425.

[0123] In particular embodiments, the adenosine deaminase comprises a V106Q mutation and an N108W mutation in SEQ ID NO: 425. In particular embodiments, the adenosine deaminase comprises a V106W mutation, an N108W mutation and an R47Z mutation, wherein Z is selected from the residues consisting of Q, F, W and M, in SEQ ID NO: 425.

[0124] It should be appreciated that any of the mutations provided herein (e.g., based on the ecTadA amino acid sequence of SEQ ID NO: 422) may be introduced into other adenosine deaminases, such as *S. aureus* TadA (saTadA), or other adenosine deaminases (e.g., bacterial adenosine deaminases), such as those sequences provided below. It would be apparent to the skilled artisan how to identify amino acid residues from other adenosine deaminases that are homologous to the mutated residues in ecTadA. Thus, any of the mutations identified in ecTadA may be made in

other adenosine deaminases that have homologous amino acid residues. It should also be appreciated that any of the mutations provided herein may be made individually or in any combination in ecTadA or another adenosine deaminase. For example, an adenosine deaminase may contain a D108N, an A106V, and/or a R47Q mutation in ecTadA SEQ ID NO: 422, or a corresponding mutation in another adenosine deaminase.

[0125] In some embodiments, the adenosine deaminase comprises one, two, or three mutations selected from the group consisting of D108, A106, and R47 in SEQ ID NO: 422, or a corresponding mutation or mutations in another adenosine deaminase.

[0126] In other aspects, the disclosure provides adenine base editors with broadened target sequence compatibility. In general, native ecTadA deaminates the adenine in the sequence UAC (e.g., the target sequence) of the anticodon loop of tRNAArg. Without wishing to be bound by any particular theory, in order to expand the utility of ABEs comprising one or more ecTadA deaminases, such as any of the adenosine deaminases provided herein, the adenosine deaminase proteins were optimized to recognize a wide variety of target sequences within the protospacer sequence without compromising the editing efficiency of the adenosine nucleobase editor complex. In some embodiments, the target sequence is an A in the middle of a 5'-NAN-3' sequence, wherein N is T, C, G, or A. In some embodiments, the target sequence comprises 5'-TAC-3'. In some embodiments, the target sequence comprises 5'-GAA-3'.

[0127] In some embodiments, the adenosine deaminase is an N-terminal truncated *E. coli* TadA. In certain embodiments, the adenosine deaminase comprises the amino acid sequence:

(SEQ ID NO: 413)
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPI

GRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSR

IGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSD

FFRMRRQEIKAQKKAQSSTD.

[0128] In some embodiments, the TadA deaminase is a full-length *E. coli* TadA deaminase (ecTadA). For example, in certain embodiments, the adenosine deaminase comprises the amino acid sequence:

(SEQ ID NO: 414)
MRRAFITGVFFLSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNN

RVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPC

VMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVLHHPGMNHRVEITEGI

LADECAALLSDFFRMRRQEIKAQKKAQSSTD

[0129] It should be appreciated, however, that additional adenosine deaminases useful in the present application would be apparent to the skilled artisan and are within the scope of this disclosure. For example, the adenosine deaminase may be a homolog of an ADAT. Exemplary ADAT homologs include, without limitation:

Staphylococcus aureus TadA:
(SEQ ID NO: 415)
MGSHMTNDIYFMTLAIEEAKKAAQLGEVPIGAIITKDDEVIARAHNLRETL

QQPTAHAEHIAIERAAKVLGSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADD

PKGGCSGSLMNLLQQSNFNHRAIVDKGVLKEACSTLLTTFFKNLRANKKSTN

Bacillus subtilis TadA:
(SEQ ID NO: 416)
MTQDELYMKEAIKEAKKAEEKGEVPIGAVLVINGEIIARAHNLRETEQRSI

AHAEMLVIDEACKALGTWRLEGATLYVTLEPCPMCAGAVVLSRVEKVVFGAFDPK

GGCSGTLMNLLQEERFNHQAEVVSGVLEEECGGMLSAFFRELRKKKKAARKNLSE

Salmonella typhimurium (*S. typhimurium*) TadA:
(SEQ ID NO: 417)
MPPAFITGVTSLSDVELDHEYWMRHALTLAKRAWDEREVPVGAVLVHNH

RVIGEGWNRPIGRHDPTAHAEIMALRQGGLVLQNYRLLDTTLYVTLEPCVMCAGAM

VHSRIGRVVFGARDAKTGAAGSLIDVLHHPGMNHRVEIIEGVLRDECATLLSDFFRM

RRQEIKALKKADRAEGAGPAV

Shewanella putrefaciens (*S. putrefaciens*) TadA:
(SEQ ID NO: 418)
MDEYWMQVAMQMAEKAEAAGEVPVGAVLVKDGQQIATGYNLSISQHDP

TAHAEILCLRSAGKKLENYRLLDATLYITLEPCAMCAGAMVHSRIARVVYGARDEK

TGAAGTVVNLLQHPAFNHQVEVTSGVLAEACSAQLSRFFKRRRDEKKALKLAQRAQ

QGIE

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Haemophilus influenzae F3031 (*H. influenzae*) TadA:
(SEQ ID NO: 419)
MDAAKVRSEFDEKMMRYALELADKAEALGEIPVGAVLVDDARNIIGEGW

NLSIVQSDPTAHAEIIALRNGAKNIQNYRLLNSTLYVTLEPCTMCAGAILHSRIKRLVF

GASDYKTGAIGSRFHFFDDYKMNHTLEITSGVLAEECSQKLSTFFQKRREEKKIEKAL

LKSLSDK

Caulobacter crescentus (*C. crescentus*) TadA:
(SEQ ID NO: 420)
MRTDESEDQDHRMMRLALDAARAAAEAGETPVGAVILDPSTGEVIATAG

NGPIAAHDPTAHAEIAAMRAAAAKLGNYRLTDLTLVVTLEPCAMCAGAISHARIGR

VVFGADDPKGGAVVHGPKFFAQPTCHWRPEVTGGVLADESADLLRGFFRARRKAKI

Geobacter sulfurreducens (*G. sulfurreducens*) TadA:
(SEQ ID NO: 421)
MSSLKKTPIRDDAYWMGKAIREAAKAAARDEVPIGAVIVRDGAVIGRGHN

LREGSNDPSAHAEMIAIRQAARRSANWRLTGATLYVTLEPCLMCMGAIILARLERVV

FGCYDPKGGAAGSLYDLSADPRLNHQVRLSPGVCQEECGTMLSDFFRDLRRRKKAK

ATPALFIDERKVPPEP

[0130] Exemplary adenosine deaminase variants of the disclosure are described below. In certain embodiments, the adenosine deaminase has a sequence with at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 99.5% sequence identity to one of the following:

(Ec)TadA, catalytically inactive (E59A)
(SEQ ID NO: 422)
MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPI

GRHDPTAHAAIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFG

ARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKK

AQSSTD

TadA 7.10
(SEQ ID NO: 423)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (V106W)
(SEQ ID NO: 424)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

WRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (N108W)
(SEQ ID NO: 425)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRWAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

-continued

TadA 7.10 (N108Q)
(SEQ ID NO: 426)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRQAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (V106F)
(SEQ ID NO: 427)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

FRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQKK

AQSSTD

TadA 7.10 (V106Q)
(SEQ ID NO: 428)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

QRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (V106M)
(SEQ ID NO: 429)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNRAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

MRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (R47F)
(SEQ ID NO: 430)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNFAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (R47W)
(SEQ ID NO: 431)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNWAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA 7.10 (R47Q)
(SEQ ID NO: 432)
MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNQAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

-continued

TadA 7.10 (R47M)

(SEQ ID NO: 433)

MSEVEFSHEYWMRHALTLAKRARDEREVPVGAVLVLNNRVIGEGWNMAI

GLHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTFEPCVMCAGAMIHSRIGRVVFG

VRNAKTGAAGSLMDVLHYPGMNHRVEITEGILADECAALLCYFFRMPRQVFNAQK

KAQSSTD

TadA (E59Q)

(SEQ ID NO: 434)

MSEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPI

GRHDPTAHAQIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFG

ARDAKTGAAGSLMDVLHHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKK

AQSSTD

[0131] Any two or more of the adenosine deaminases described herein may be connected to one another (e.g. by a linker) within an adenosine deaminase domain of the fusion proteins provided herein. For instance, the fusion proteins provided herein may contain only two adenosine deaminases. In some embodiments, the adenosine deaminases are the same. In some embodiments, the adenosine deaminases are any of the adenosine deaminases provided herein. In some embodiments, the adenosine deaminases are different. In some embodiments, the first adenosine deaminase is any of the adenosine deaminases provided herein, and the second adenosine is any of the adenosine deaminases provided herein, but is not identical to the first adenosine deaminase. In some embodiments, the fusion protein comprises two adenosine deaminases (e.g., a first adenosine deaminase and a second adenosine deaminase). In some embodiments, the fusion protein comprises a first adenosine deaminase and a second adenosine deaminase. In some embodiments, the first adenosine deaminase is N-terminal to the second adenosine deaminase in the fusion protein. In some embodiments, the first adenosine deaminase is C-terminal to the second adenosine deaminase in the fusion protein. In some embodiments, the first adenosine deaminase and the second deaminase are fused directly or via a linker.

[0132] In particular embodiments, the base editors disclosed herein comprise a heterodimer of a first adenosine deaminase that is N-terminal to a second adenosine deaminase, wherein the first adenosine deaminase comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO: 413-434; and the second adenosine deaminase comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO: 413-434.

[0133] In other embodiments, the second adenosine deaminase of the base editors provided herein comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO: 423 (TadA 7.10), wherein any sequence variation may only occur in amino acid positions other than R47, V106 or N108 of SEQ ID NO: 423. In other words, these embodiments must contain amino acid substitutions at R47, V106 or N108 of SEQ ID NO: 423.

[0134] In other embodiments, the second adenosine deaminase of the heterodimer comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, 99%, or 99.5% sequence identity to SEQ ID NO: 413-434.

F. Recombinases

[0135] In other embodiments, the GeoCas9 base editors may comprise a recombinase as the nucleic acid effector domain.

[0136] The term "recombinase," as used herein, refers to a site-specific enzyme that mediates the recombination of DNA between recombinase recognition sequences, which results in the excision, integration, inversion, or exchange (e.g., translocation) of DNA fragments between the recombinase recognition sequences. Recombinases can be classified into two distinct families: serine recombinases (e.g., resolvases and invertases) and tyrosine recombinases (e.g., integrases). Examples of serine recombinases include, without limitation, Hin, Gin, Tn3, β-six, CinH, ParA, γδ, Bxb1, ϕC31, TP901, TG1, φBT1, R4, φRV1, φFC1, MR11, A118, U153, and gp29. Examples of tyrosine recombinases include, without limitation, Cre, FLP, R, Lambda, HK101, HK022, and pSAM2. The Gin recombinase referred to herein may be any Gin recombinase known in the art including, but not limited to, the Gin recombinases presented in T. Gaj et al., A comprehensive approach to zinc-finger recombinase customization enables genomic targeting in human cells. *Nucleic Acids Research* 41, 3937-3946 (2013), incorporated herein by reference in its entirety. In certain embodiments, the Gin recombinase catalytic domain has greater than 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence shown in SEQ ID NO: 384. In another embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises a mutation corresponding to H106Y, and/or I127L, and/or I136R and/or G137F. In yet another embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises a mutation corresponding to H106Y, I127L, 1136R, and G137F.

[0137] In a further embodiment, the amino acid sequence of the Gin recombinase has been further mutated. In a specific embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises SEQ ID NO: 384. Gin recombinases bind to gix target sites (also referred to herein as "gix core," "minimal gix core," or "gix-related core" sequences). The minimal gix core recombinase site is NNNNAAASSWWSSTTTNNNN (SEQ ID NO: 384), wherein N is defined as any amino acid, W is an A or a T,

and S is a G or a C. The gix target site may include any other mutations known in the art. In certain embodiments, the gix target site has greater than 90%, 95%, or 99% sequence identity with the amino acid sequence shown in SEQ ID NO: 384. The distance between the gix core or gix-related core sequence and at least one gRNA binding site may be from 1 to 10 base pairs, from 3 to 7 base pairs, from 5 to 7 base pairs, or from 5 to 6 base pairs. The distance between the gix core or gix-related core sequence and at least one gRNA binding site may be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 base pairs.

[0138] The serine and tyrosine recombinase names stem from the conserved nucleophilic amino acid residue that the recombinase uses to attack the DNA and which becomes covalently linked to the DNA during strand exchange. Recombinases have numerous applications, including the creation of gene knockouts/knock-ins and gene therapy applications. See, e.g., Brown et al., "Serine recombinases as tools for genome engineering." *Methods.* 2011; 53 (4): 372-9; Hirano et al., "Site-specific recombinases as tools for heterologous gene integration." *Appl. Microbiol. Biotechnol.* 2011; 92 (2): 227-39; Chavez and Calos, "Therapeutic applications of the ΦC31 integrase system." *Curr. Gene Ther.* 2011; 11 (5): 375-81; Turan and Bode, "Site-specific recombinases: from tag-and-target-to tag-and-exchange-based genomic modifications." *FASEB J.* 2011; 25 (12): 4088-107; Venken and Bellen, "Genome-wide manipulations of *Drosophila melanogaster* with transposons, Flp recombinase, and @C31 integrase." *Methods Mol. Biol.* 2012; 859:203-28; Murphy, "Phage recombinases and their applications." *Adv. Virus Res.* 2012; 83:367-414; Zhang et al., "Conditional gene manipulation: Creating a new biological era." *J. Zhejiang Univ. Sci. B.* 2012; 13 (7): 511-24; Karpenshif and Bernstein, "From yeast to mammals: recent advances in genetic control of homologous recombination." *DNA Repair* (*Amst*). 2012; 1; 11 (10): 781-8; the entire contents of each are hereby incorporated by reference in their entirety. The recombinases provided herein are not meant to be exclusive examples of recombinases that can be used in embodiments of the invention. The methods and compositions of the invention can be expanded by mining databases for new orthogonal recombinases or designing synthetic recombinases with defined DNA specificities (See, e.g., Groth et al., "Phage integrases: biology and applications." *J. Mol. Biol.* 2004; 335, 667-678; Gordley et al., "Synthesis of programmable integrases." *Proc. Natl. Acad. Sci. USA.* 2009; 106, 5053-5058; the entire contents of each are hereby incorporated by reference in their entirety).

[0139] Other examples of recombinases that are useful in the methods and compositions described herein are known to those of skill in the art, and any new recombinase that is discovered or generated is expected to be able to be used in the different embodiments of the invention. In some embodiments, the catalytic domains of a recombinase are fused to a nuclease-inactivated RNA-programmable nuclease (e.g., dCas9, or a fragment thereof), such that the recombinase domain does not comprise a nucleic acid binding domain or is unable to bind to a target nucleic acid that subsequently results in enzymatic catalysis (e.g., the recombinase domain is engineered such that it does not have specific DNA binding activity). Recombinases lacking part of their DNA binding activity and those that act independently of accessory proteins and methods for engineering such are known, and include those described by Klippel et al., "Isolation and characterisation of unusual gin mutants." *EMBO J.* 1988; 7:3983-3989: Burke et al., "Activating mutations of Tn3 resolvase marking interfaces important in recombination catalysis and its regulation. *Mol Microbiol.* 2004; 51:937-948; Olorunniji et al., "Synapsis and catalysis by activated Tn3 resolvase mutants." *Nucleic Acids Res.* 2008; 36:7181-7191; Rowland et al., "Regulatory mutations in Sin recombinase support a structure-based model of the synaptosome." *Mol Microbiol.* 2009; 74:282-298; Akopian et al., "Chimeric recombinases with designed DNA sequence recognition." *Proc Natl Acad Sci USA.* 2003; 100:8688-8691; Gordley et al., "Evolution of programmable zinc finger-recombinases with activity in human cells. *J Mol Biol.* 2007; 367:802-813; Gordley et al., "Synthesis of programmable integrases." *Proc Natl Acad Sci USA.* 2009; 106:5053-5058; Arnold et al., "Mutants of Tn3 resolvase which do not require accessory binding sites for recombination activity." *EMBO J.* 1999; 18:1407-1414; Gaj et al., "Structure-guided reprogramming of serine recombinase DNA sequence specificity. " *Proc Natl Acad Sci USA.* 2011; 108 (2): 498-503; and Proudfoot et al., "Zinc finger recombinases with adaptable DNA sequence specificity." *PLOS One.* 2011; 6 (4): e19537; the entire contents of each are hereby incorporated by reference.

[0140] For example, serine recombinases of the resolvase-invertase group, e.g., Tn3 and γδ resolvases and the Hin and Gin invertases, have modular structures with partly autonomous catalytic and DNA-binding domains (See, e.g., Grindley et al., "Mechanism of site-specific recombination." *Ann Rev Biochem.* 2006; 75:567-605, the entire contents of which are incorporated by reference). The catalytic domains of these recombinases are therefore amenable to being recombined with nuclease-inactivated RNA-programmable nucleases (e.g., dCas9, or a fragment thereof) as described herein, e.g., following the isolation of 'activated' recombinase mutants which do not require any accessory factors (e.g., DNA binding activities) (See, e.g., Klippel et al., "Isolation and characterisation of unusual gin mutants." *EMBO J.* 1988; 7:3983-3989: Burke et al., "Activating mutations of Tn3 resolvase marking interfaces important in recombination catalysis and its regulation. *Mol Microbiol.* 2004; 51:937-948; Olorunniji et al., "Synapsis and catalysis by activated Tn3 resolvase mutants." *Nucleic Acids Res.* 2008; 36:7181-7191; Rowland et al., "Regulatory mutations in Sin recombinase support a structure-based model of the synaptosome." *Mol Microbiol.* 2009; 74:282-298; Akopian et al., "Chimeric recombinases with designed DNA sequence recognition." *Proc Natl Acad Sci USA.* 2003; 100:8688-8691). The GeoCas9 base editor fusion proteins contemplated herein may comprise any of these recombinase proteins.

[0141] Additionally, many other natural serine recombinases having an N-terminal catalytic domain and a C-terminal DNA binding domain are known (e.g., phiC31 integrase, TnpX transposase, IS607 transposase), and their catalytic domains can be co-opted to engineer programmable site-specific recombinases as described herein (See, e.g., Smith et al., "Diversity in the serine recombinases." *Mol Microbiol.* 2002; 44:299-307, the entire contents of which are incorporated by reference). Similarly, the core catalytic domains of tyrosine recombinases (e.g., Cre, 2 integrase) are known, and can be similarly co-opted to engineer programmable site-specific recombinases as described herein (See, e.g., Guo et al., "Structure of Cre recombinase complexed with DNA in a site-specific recom-

bination synapse." *Nature.* 1997; 389:40-46; Hartung et al., "Cre mutants with altered DNA binding properties." *J Biol Chem* 1998; 273:22884-22891; Shaikh et al., "Chimeras of the Flp and Cre recombinases: Tests of the mode of cleavage by Flp and Cre. *J Mol Biol.* 2000; 302:27-48; Rongrong et al., "Effect of deletion mutation on the recombination activity of Cre recombinase." *Acta Biochim Pol.* 2005; 52:541-544; Kilbride et al., "Determinants of product topology in a hybrid Cre-Tn3 resolvase site-specific recombination system." *J Mol Biol.* 2006; 355:185-195; Warren et al., "A chimeric cre recombinase with regulated directionality." *Proc Natl Acad Sci USA.* 2008 105:18278-18283; Van Duyne, "Teaching Cre to follow directions." *Proc Natl Acad Sci USA.* 2009 Jan. 6; 106 (1): 4-5; Numrych et al., "A comparison of the effects of single-base and triple-base changes in the integrase arm-type binding sites on the site-specific recombination of bacteriophage 2." *Nucleic Acids Res.* 1990; 18:3953-3959; Tirumalai et al., "The recognition of core-type DNA sites by λ integrase." *J Mol Biol.* 1998; 279:513-527; Aihara et al., "A conformational switch controls the DNA cleavage activity of A integrase." *Mol Cell.* 2003; 12:187-198; Biswas et al., "A structural basis for allosteric control of DNA recombination by λ integrase." *Nature.* 2005; 435:1059-1066; and Warren et al., "Mutations in the amino-terminal domain of λ-integrase have differential effects on integrative and excisive recombination." *Mol Microbiol.* 2005; 55:1104-1112; the entire contents of each are incorporated by reference). The Geo-Cas9 base editor fusion proteins contemplated herein may comprise any of these recombinase proteins.

[0142] The recombinase catalytic domain for use in the compositions and methods of the instant disclosure may be from any recombinase. Suitable recombinases catalytic domains for use in the disclosed methods and compositions may be obtained from, for example, and without limitation, tyrosine recombinases and serine recombinases. Some exemplary suitable recombinases provided herein include, for example, and without limitation, Gin recombinase (acting on gix sites), Hin recombinase (acting on hix sites), β recombinase (acting on six sites), Sin recombinase (acting on resH sites), Tn3 recombinase (acting on res sites), γδ recombinase (acting on res sites), Cre recombinase from bacteriophage P1 (acting on LoxP sites); FLP recombinases of fungal origin (acting on FTR sites); and phiC31 integrase (acting on att sites). Non-limiting sequences of exemplary suitable recombinases may be found below.

Cre recombinase

(SEQ ID NO: 385)

MSNLLTVHQNLPALPVDATSDEVRKNLMDMFRDRQAFSEHTWKMLLSVC

RSWAAWCKLNNRKWFPAEPEDVRDYLLYLQARGLAVKTIQQHLGQLNMLHRRSGL

PRPSDSNAVSLVMRRIRKENVDAGERAKQALAFERTDFDQVRSLMENSDRCQDIRN

LAFLGIAYNTLLRIAEIARIRVKDISRTDGGRMLIHIGRTKTLVSTAGVEKALSLGVTK

LVERWISVSGVADDPNNYLFCRVRKNGVAAPSATSQLSTRALEGIFEATHRLIYGAK

DDSGQRYLAWSGHSARVGAARDMARAGVSIPEIMQAGGWTNVNIVMNYIRNLDSE

TGAMVRLLEDGD.

FLP recombinase

(SEQ ID NO: 386)

MPQFGILCKTPPKVLVRQFVERFERPSGEKIALCAAELTYLCWMITHNGTAI

KRATFMSYNTIISNSLSFDIVNKSLQFKYKTQKATILEASLKKLIPAWEFTIIPYYGQKH

QSDITDIVSSLQLQFESSEEADKGNSHSKKMLKALLSEGESIWEITEKILNSFEYTSRFT

KTKTLYQFLFLATFINCGRFSDIKNVDPKSFKLVQNKYLGVIIQCLVTETKTSVSRHIY

FFSARGRIDPLVYLDEFLRNSEPVLKRVNRTGNSSSNKQEYQLLKDNLVRSYNKALK

KNAPYSIFAIKNGPKSHIGRHLMTSFLSMKGLTELTNVVGNWSDKRASAVARTTYTH

QITAIPDHYFALVSRYYAYDPISKEMIALKDETNPIEEWQHIEQLKGSAEGSIRYPAW

NGIISQEVLDYLSSYINRRI.

γδ recombinase (Gamma Delta resolvase)

(SEQ ID NO: 387)

MRLFGYARVSTSQQSLDIQVRALKDAGVKANRIFTDKASGSSSDRKGLDL

LRMKVEEGDVILVKKLDRLGRDTADMIQLIKEFDAQGVSIRFIDDGISTDGEMGKMV

VTILSAVAQAERQRILERTNEGRQEAMAKGVVFGRKR

-continued

γδ recombinase (E124Q mutation)
(SEQ ID NO: 388)
MRLFGYARVSTSQQSLDIQVRALKDAGVKANRIFTDKASGSSSDRKGLDL

LRMKVEEGDVILVKKLDRLGRDTADMIQLIKEFDAQGVSIRFIDDGISTDGEMGKMV

VTILSAVAQAERQRILQRTNEGRQEAMAKGVVFGRKR.

γδ recombinase (E102Y/E124Q mutation)
(SEQ ID NO: 389)
MRLFGYARVSTSQQSLDIQVRALKDAGVKANRIFTDKASGSSSDRKGLDL

LRMKVEEGDVILVKKLDRLGRDTADMIQLIKEFDAQGVSIRFIDDGISTDGYMGKMV

VTILSAVAQAERQRILQRTNEGRQEAMAKGVVFGRKR.

β recombinase
(SEQ ID NO: 390)
MAKIGYARVSSKEQNLDRQLQALQGVSKVFSDKLSGQSVERPQLQAMLN

YIREGDIVVVTELDRLGRNNKELTELMNAIQQKGATLEVLDLPSMNGIEDENLRRLIN

NLVIELYKYQAESERKRIKERQAQGIEIAKSKGKFKGRQH.

β recombinase (N95D mutation)
(SEQ ID NO: 391)
MAKIGYARVSSKEQNLDRQLQALQGVSKVFSDKLSGQSVERPQLQAMLNYIREGDI

VVVTELDRLGRNNKELTELMNAIQQKGATLEVLDLPSMDGIEDENLRRLINNLVIEL

YKYQAESERKRIKERQAQGIEIAKSKGKFKGRQH.

Sin recombinase
(SEQ ID NO: 392)
MIIGYARVSSLDQNLERQLENLKTFGAEKIFTEKQSGKSIENRPILQKALNF

VRMGDRFIVESIDRLGRNYNEVIHTVNYLKDKEVQLMITSLPMMNEVIGNPLLDKFM

KDLIIQILAMVSEQERNESKRRQAQGIQVAKEKGVYKGRPL.

Sin recombinase (Q87R/Q115R mutations)
(SEQ ID NO: 393)
MIIGYARVSSLDQNLERQLENLKTFGAEKIFTEKQSGKSIENRPILQKALNF

VRMGDRFIVESIDRLGRNYNEVIHTVNYLKDKEVRLMITSLPMMNEVIGNPLLDKFM

KDLIIRILAMVSEQERNESKRRQAQGIQVAKEKGVYKGRPL.

Tn3 recombinase
(SEQ ID NO: 394)
MRLFGYARVSTSQQSLDLQVRALKDAGVKANRIFTDKASGSSTDREGLDL

LRMKVKEGDVILVKKLDRLGRDTADMLQLIKEFDAQGVAVRFIDDGISTDGDMGQ

MVVTILSAVAQAERRRILERTNEGRQEAKLKGIKFGRRR.

Tn3 recombinase (G70S/D102Y, E124Q mutations)
(SEQ ID NO: 395)
MRLFGYARVSTSQQSLDLQVRALKDAGVKANRIFTDKASGSSTDREGLDL

LRMKVKEGDVILVKKLDRLSRDTADMLQLIKEFDAQGVAVRFIDDGISTDGYMGQM

VVTILSAVAQAERRRILQRTNEGRQEAKLKGIKFGRRR.

Hin recombinase
(SEQ ID NO: 396)
MATIGYIRVSTIDQNIDLQRNALTSANCDRIFEDRISGKIANRPGLKRALKY

VNKGDTLVVWKLDRLGRSVKNLVALISELHERGAHFHSLTDSIDTSSAMGRFFFHV

MSALAEMERELIVERTLAGLAAARAQGRLGGRPV.

-continued

Hin recombinase (H107Y mutation)

(SEQ ID NO: 397)

MATIGYIRVSTIDQNIDLQRNALTSANCDRIFEDRISGKIANRPGLKRALKY

VNKGDTLVVWKLDRLGRSVKNLVALISELHERGAHFHSLTDSIDTSSAMGRFFFYV

MSALAEMERELIVERTLAGLAAARAQGRLGGRPV.

PhiC31 recombinase

(SEQ ID NO: 398)

MDTYAGAYDRQSRERENSSAASPATQRSANEDKAADLQREVERDGGRFR

FVGHFSEAPGTSAFGTAERPEFERILNECRAGRLNMIIVYDVSRFSRLKVMDAIPIVSE

LLALGVTIVSTQEGVFRQGNVMDLIHLIMRLDASHKESSLKSAKILDTKNLQRELGG

YVGGKAPYGFELVSETKEITRNGRMVNVVINKLAHSTTPLTGPFEFEPDVIRWWWRE

IKTHKHLPFKPGSQAAIHPGSITGLCKRMDADAVPTRGETIGKKTASSAWDPATVMR

ILRDPRIAGFAAEVIYKKKPDGTPTTKIEGYRIQRDPITLRPVELDCGPIIEPAEWYELQ

AWLDGRGRGKGLSRGQAILSAMDKLYCECGAVMTSKRGEESIKDSYRCRRRKVVD

PSAPGQHEGTCNVSMAALDKFVAERIFNKIRHAEGDEETLALLWEAARRFGKLTEAP

EKSGERANLVAERADALNALEELYEDRAAGAYDGPVGRKHFRKQQAALTLRQQGA

EERLAELEAAEAPKLPLDQWFPEDADADPTGPKSWWGRASVDDKRVFVGLFVDKIV

VTKSTTGRGQGTPIEKRASITWAKPPTDDDEDDAQDGTEDVAATGA.

[0143] Recombinases for use with the GeoCas9 base editor fusion proteins may also include further mutations. Some aspects of this disclosure provide recombinases comprising an amino acid sequence that is at least 70%, at least 80%, at least 90%, at least 95%, or at least 97% identical to the sequence of the recombinase sequence discussed herein, wherein the amino acid sequence of the recombinase comprises at least one mutation as compared to the sequence of the recombinase sequence discussed herein. In some embodiments, the amino acid sequence of the recombinase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 mutations as compared to the sequence of the recombinase sequence discussed herein.

[0144] For example, the γδ recombinase may comprise one or more mutations from the list: R2A, E56K, G101S, E102Y, M103I, or E124Q. In one embodiment, the γδ recombinase may comprise an E102Y mutation, an E124Q mutation, or both an E102Y and E124Q mutation. In another embodiment, the β recombinase may comprise one or more mutations including, but not limited to N95D. See, for example, Sirk et al., "Expanding the zinc-finger recombinase repertoire: directed evolution and mutational analysis of serine recombinase specificity determinants" Nucl Acids Res (2014) 42 (7): 4755-4766. In another embodiment, the Sin recombinase may have one or more mutations including, but not limited to: Q87R, Q115R, or Q87R and Q115R. In another embodiment, the Tn3 recombinase may have one or more mutations including, but not limited to: G70S, D102Y, E124Q, and any combination thereof. In another embodiment, the Hin recombinase may have one or more mutations including, but not limited to: H107Y. In another embodiment, the Sin recombinase may have one or more mutations including, but not limited to: H107Y. Any of the recombinase catalytic domains for use with the disclosed compositions and methods may have greater than 85%, 90%, 95%, 98%, or 99% sequence identity with the native (or wild type) amino acid sequence. For example, in certain embodiments, the Gin recombinase catalytic domain has greater than 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence shown in SEQ ID NO: 384. In another embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises a mutation corresponding to H106Y, and/or I127L, and/or I136R and/or G137F. In yet another embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises a mutation corresponding to H106Y, I127L, 1136R, and G137F. In a further embodiment, the amino acid sequence of the Gin recombinase has been further mutated. In a specific embodiment, the amino acid sequence of the Gin recombinase catalytic domain comprises SEQ ID NO: 384.

[0145] In some embodiments, only a portion of the recombinase is used in the GeoCas9 fusion proteins and methods described herein. As a non-limiting embodiment, only the C-terminal portion of the recombinase may be used in the fusion proteins and methods described herein. In a specific embodiment, the 25 kDa carboxy-terminal domain of Cre recombinase may be used in the compositions and methods. See, for example, Hoess et al, "DNA Specificity of the Cre Recombinase Resides in the 25 kDa Carboxyl Domain of the Protein," J. Mol. Bio. 1990 Dec. 20, 216 (4): 873-82, which is incorporated by reference herein for all purposes. The 25 kDa carboxy-terminal domain of Cre recombinase is the portion stretching from R118 to the carboxy terminus of the protein. In some embodiments, the 25 kDa carboxy-terminal domain of Cre recombinase for use in the instant fusion proteins and methods may differ from the canonical 25 kDa carboxy-terminal domain of Cre recombinase by at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at

least 19, or at least 20 amino acids. In some embodiments, the 25 kDa carboxy-terminal domain of Cre recombinase for use in the instant fusion proteins and methods may differ from the canonical 25 kDa carboxy-terminal domain of Cre recombinase by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids. In certain embodiments, only a portion of the 25 kDa carboxy-terminal domain of Cre recombinase may be used in the fusion proteins and methods described herein. For example, the portion of Cre recombinase used may be R130 to the carboxy terminus of the protein, T140 to the carboxy terminus of the protein, E150 to the carboxy terminus of the protein, N160 to the carboxy terminus of the protein, T170 to the carboxy terminus of the protein, 1180 to the carboxy terminus of the protein, G190 to the carboxy terminus of the protein, T200 to the carboxy terminus of the protein, E210 to the carboxy terminus of the protein, L220 to the carboxy terminus of the protein, V230 to the carboxy terminus of the protein, C240 to the carboxy terminus of the protein, P250 to the carboxy terminus of the protein, A260 to the carboxy terminus of the protein, R270 to the carboxy terminus of the protein, G280 to the carboxy terminus of the protein, S290 to the carboxy terminus of the protein, A300 to the carboxy terminus of the protein, or M310 to the carboxy terminus of the protein. As another set of non-limiting examples, the portion of Cre recombinase used may be R118-E340, R118-S330, R118-1320, R118-M310, R118-A300, R118-S290, R118-G280, R118-R270, R118-A260, R118-P250, R118-C240, R118-V230, R118-L220, or R118-E210. As a further set of non-limiting examples, the portion of Cre recombinase used may be R118-E210, G190-R270, E210-S290, P250-M310, or R270 to the carboxy terminus of the protein.

[0146] In some embodiments, the Cre recombinase used in the fusion proteins and methods described herein may be truncated at any position. In a specific embodiment, the Cre recombinase used in the fusion proteins and methods described herein may be truncated such that it begins with amino acid R118, A127, E138, or R154) (preceded in each case by methionine). In another set of non-limiting embodiments, the Cre recombinase used in the fusion proteins and methods described herein may be truncated within 10 amino acids, 9 amino acids, 8 amino acids, 7 amino acids, 6 amino acids, 5 amino acids, 4 amino acids, 3 amino acids, 2 amino acids, or 1 amino acid of R118, A127, E138, or R154.

[0147] In some embodiments, the recombinase target sequence is between 10-50 nucleotides long. In some embodiments, the recombinase is a Cre recombinase, a Hin recombinase, or a FLP recombinase. In some embodiments, the canonical recombinase target sequence is a LoxP site (5'-ATAACTTCGTATA GCATACAT TATACGAAGTTAT-3' (SEQ ID NO: 399). In some embodiments, the canonical recombinase target sequence is an FRT site (5'-GAAGTTCCTATTCTCTAGAAA GTATAGGAACTTC-3') (SEQ ID NO: 400). In some embodiments, the amino acid sequence of the evolved recombinase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 mutations as compared to the sequence of the wild-type recombinase. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence that comprises a left half-site, a spacer sequence, and a right half-site, and wherein the left half-site is not a palindrome of the right half-site.

[0148] In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence that comprises a naturally occurring sequence. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence that is comprised in the genome of a mammal. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence comprised in the genome of a human. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence that occurs only once in the genome of a mammal. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence in the genome of a mammal that differs from any other site in the genome by at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 nucleotide(s). In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence located in a safe harbor genomic locus. In some embodiments, the safe harbor genomic locus is a Rosa26 locus. In some embodiments, the evolved recombinase recognizes a DNA recombinase target sequence located in a genomic locus associated with a disease or disorder.

[0149] In certain embodiments, the evolved recombinase may target a site in the Rosa locus of the human genome (e.g., 36C6). A non-limiting set of such recombinases may be found, for example, in International PCT Publication, WO 2017/015545A1, published Jan. 26, 2017, entitled "Evolution of Site Specific Recombinases," which is incorporated by reference herein for this purpose. In some embodiments, the amino acid sequence of the evolved recombinase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, or at least 15 mutations as compared to the sequence of the wild-type recombinase. The nucleotide sequence encoding 36C6 is shown below in bold; those encoding GGS linkers are shown in italics; those encoding dCas9 linkers are black; those encoding the FLAG tag and NLS are underlined and in lowercase, respectively.

G. Other Effector Domains

[0150] In addition to cytidine deaminases, adenosine deaminasws GeoCas9 base editor fusion proteins may comprise one or more nucleic acid effector domains. Exemplary nucleic acid effector domains include, but are not limited to a deaminase (e.g., a cytidine deaminase or an adenosine deaminase), a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain. In some embodiments the nucleic acid effector domain is a protein or enzyme capable of making one or more modifications (e.g., deamination of a cytidine residue) to a nucleic acid (e.g., DNA or RNA). In some embodiments the nucleic acid editing domain is a deaminase (e.g., a cytidine deaminase, such as an APOBEC or an AID deaminase). Additional suitable nucleic-acid effector enzyme sequences that can be used according to aspects of this invention, e.g., that can be fused to a GeoCas9 protein, will be apparent to those of skill in the art based on this disclosure. In some embodiments, such additional enzyme sequences include deaminase enzyme or deaminase domain sequences that are at least 70%, at least 75%, at least 80%,

at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% similar to the sequences provided herein.

[0151] In some embodiments, the GeoCas9 base editors may comprise a Gam protein as the nucleic acid effector domain.

[0152] The term "Gam proteins," as used herein, refers generally to proteins capable of binding to one or more ends of a double strand break of a double stranded nucleic acid (e.g., double stranded DNA). In some embodiments, the Gam protein prevents or inhibits degradation of one or more strands of a nucleic acid at the site of the double strand break. In some embodiments, a Gam protein is a naturally-occurring Gam protein from bacteriophage Mu, or a non-naturally occurring variant thereof. Gam Proteins are described in PCT Publication No. WO2019/139645, the contents of each of which are incorporated herein by reference.

[0153] Gam is related to Ku70 and Ku80, two eukaryotic proteins involved in non-homologous DNA end-joining (Cui et al., 2016). Gam has sequence homology with both subunits of Ku (Ku70 and Ku80), and can have a similar structure to the core DNA-binding region of Ku. Orthologs to Mu Gam are present in the bacterial genomes of *Haemophilus influenzae, Salmonella typhi, Neisseria meningitidis* and the enterohemorrhagic 0157: H7 strain of *E. coli* (d'Adda di Fagagna et al., 2003). Gam proteins have been described previously, for example, in COX, Proteins pinpoint double strand breaks. eLife.2013; 2: e01561.; CUI et al., Consequences of Cas9 cleavage in the chromosome of *Escherichia coli*. Nucleic Acids Res. 2016 May 19; 44 (9): 4243-51. doi: 10.1093/nar/gkw223. Epub 2016 Apr. 8.; D'ADDA DI FAGAGNA et al., The Gam protein of bacteriophage Mu is an orthologue of eukaryotic Ku. EMBO Rep. 2003 January; 4 (1): 47-52.; and SHEE et al., Engineered proteins detect spontaneous DNA breakage in human and bacterial cells. Elife. 2013 Oct. 29; 2: e01222. doi: 10.7554/eLife.01222; the contents of each of which are incorporated herein by reference.

[0154] In some embodiments, the Gam protein comprises the amino acid sequence of the Gam form bacteriophage Mu:

(SEQ ID NO: 401)
AKPAKRIKSAAAAYVPQNRDAVITDIKRIGDLQREASRLETEMNDAIAEI

TEKFAARIAPIKTDIETLSKGVQGWCEANRDELTNGGKVKTANLVTGDVS

WRVRPPSVSIRGMDAVMETLERLGLQRFIRTKQEINKEAILLEPKAVAGV

AGITVKSGIEDFSIIPFEQEAGI

[0155] In other embodiments, the Gam protein comprises the amino acid sequences as follows:

WP_001107930.1 MULTISPECIES: host-nuclease inhibitor protein Gam [*Enterobacteriaceae*]
(SEQ ID NO: 402)
MAKPAKRIKSAAAAYVPQNRDAVITDIKRIGDLQREASRLETEMNDAIAE

ITEKFAARIAPIKTDIETLSKGVQGWCEANRDELTNGGKVKTANLVTGDV

SWRVRPPSVSIRGMDAVMETLERLGLQRFIRTKQEINKEAILLEPKAVAG

VAGITVKSGIEDFSIIPFEQEAGI

-continued

CAA27978.1 unnamed protein product [*Escherichia* virus Mu]
(SEQ ID NO: 403)
MAKPAKRIKSAAAAYVPQNRDAVITDIKRIGDLQREASRLETEMNDAIAE

ITEKFAARIAPIKTDIETLSKGVQGWCEANRDELTNGGKVKTANLVTGDV

SWRVRPPSVSIRGMDAVMETLERLGLQRFVRTKQEINKEAILLEPKAVAG

VAGITVKSGIEDFSIIPFEQEAGI

WP_001107932.1 host-nuclease inhibitor protein Gam [*Escherichia coli*]
(SEQ ID NO: 404)
MAKPAKRIKSAAAAYVPQNRDAVITDIKRIGDLQREASRLETEMNDAIAE

ITEKFAARIAPLKTDIETLSKGVQGWCEANRDELTNGGKVKTANLVTGDV

SWRVRPPSVSIRGMDAVMETLERLGLQRFIRTKQEINKEAILLEPKAVAG

VAGITVKSGIEDFSIIPFEQEAGI

WP_061335739.1 host-nuclease inhibitor protein Gam [*Escherichia coli*]
(SEQ ID NO: 405)
MAKPAKRIKSAAAAYVPQNRDAVITDIKRIGDLQREASRLETEMNDAIAE

ITEKFAARIAPIKTDIETLSKGVQGWCEANRDELTNGGKVKTANLITGDV

SWRVRPPSVSIRGMDAVMETLERLGLQRFIRTKQEINKEAILLEPKAVAG

VAGITVKSGIEDFSIIPFEQEAGI

H. Linkers

[0156] In various embodiments, the GeoCas9 base editors comprise one or more linkers that join or fuse the various functional domains of the base editors, e.g., the GeoCas9 domain and the one or more nucleic acid effector domains (e.g., a cytidine deaminase or adenosine deaminase domain).

[0157] In some embodiments, the linker comprises (GGGS)n (SEQ ID NO: 9), (GGGGS)n (SEQ ID NO: 10), (G)n (SEQ ID NO: 11), (EAAAK)n (SEQ ID NO: 12), (GGS)n (SEQ ID NO: 13), (SGGS)n (SEQ ID NO: 14), SGSETPGTSESATPES (SEQ ID NO: 15), SGGS (GGS)n (SEQ ID NO: 16), SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 17), or (XP)n (SEQ ID NO: 18) motif, or a combination of any of these, wherein n is independently an integer between 1 and 30, and wherein X is any amino acid. In some embodiments, the linker comprises a (GGS)n (SEQ ID NO: 13) motif, wherein n is 1, 3, or 7. In some embodiments, the linker comprises a (GGS)n (SEQ ID NO: 13) motif, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises the amino acid sequence SGGS (GGS)n, (SEQ ID NO: 16), wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, the linker comprises the amino acid sequence SGGS (GGS)n, (SEQ ID NO: 16), wherein n is 2. In some embodiments, the linker comprises an amino acid sequence of SGSETPGTSESATPES (SEQ ID NO: 15), also referred to as the "XTEN linker". In some embodiments, the linker comprises the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 17), also referred to as the 32 amino acid linker. The length of the linker can influence the base to be edited. For example, when the GeoCas9 is fused to a cytidine deaminase, a 16-amino-acid linker (e.g., the XTEN linker) may give a −3 to 15, a −3 to 13, a −3 to 11, a −3 to 9, a −3 to 7, a −3 to 5, a −3 to 3, a −1 to 15, a −1 to 13, a −1 to 11, a −1 to 9, a −1 to 7, a −1

to 5, a −1 to 3, a 2 to 15, a 2 to 13, a 2 to 11, a 2 to 9, a 2 to 7, a 2 to 5, a 4 to 15, a 4 to 13, a 4 to 11, a 4 to 9, a 4 to 7, a 6 to 15, a 6 to 13, a 6 to 11, or a 6 to 9, a −3 to 7, a −3 to 5, a −3 to 3, base editing window relative to the PAM sequence (e.g., a NNNNCRAA (SEQ ID NO: 79) PAM sequence having positions 21-28). As another example, when the GeoCas9 is fused to a cytidine deaminase, a 32-amino-acid linker (e.g., the (SGGS) 2XTEN (SGGS) 2 linker) (SEQ ID NO: 17) may give a −3 to 15, a −3 to 13, a −3 to 11, a −3 to 9, a −3 to 7, a −3 to 5, a −3 to 3, a −1 to 15, a −1 to 13, a −1 to 11, a −1 to 9, a −1 to 7, a −1 to 5, a −1 to 3, a 2 to 15, a 2 to 13, a 2 to 11, a 2 to 9, a 2 to 7, a 2 to 5, a 4 to 15, a 4 to 13, a 4 to 11, a 4 to 9, a 4 to 7, a 6 to 15, a 6 to 13, a 6 to 11, or a 6 to 9, a −3 to 7, a −3 to 5, a −3 to 3, base editing window relative to the PAM sequence (e.g., a NNNNCRAA (SEQ ID NO: 79) PAM sequence having positions 21-28). Varying linker length may allow GeoCas9 fusion proteins of the disclosure to edit nucleobases different distances from the PAM sequence, which affords siginicant clinical importance, since a PAM sequence may be of varying distance to the disease-causing mutation to be modified in a gene. It is to be understood that the linker lengths described as examples herein are not meant to be limiting.

[0158] Additional suitable linker motifs and linker configurations will be apparent to those of skill in the art. In some embodiments, suitable linker motifs and configurations include those described in Chen et al., Fusion protein linkers: property, design and functionality. *Adv Drug Deliv Rev.* 2013; 65 (10): 1357-69, the entire contents of which are incorporated herein by reference. Additional suitable linker sequences will be apparent to those of skill in the art based on the instant disclosure.

I. NLS and Other Sequence Tags

[0159] The GeoCas9 base editor fusion proteins of the present disclosure may also comprise one or more additional sequence features. For example, in some embodiments, the GeoCas9 base editor fusion proteins may comprise one or more nuclear localization sequences (NLS). In some embodiments, the NLS of the GeoCas9 base editor fusion proteins is localized between the nucleic acid editing domain and the GeoCas9 domain. In some embodiments, the NLS of the GeoCas9 base editor fusion protein is localized C-terminal to the GeoCas9 domain.

[0160] Other exemplary features that may be present are localization sequences, such as cytoplasmic localization sequences, export sequences, such as nuclear export sequences, or other localization sequences, as well as sequence tags that are useful for solubilization, purification, or detection of the GeoCas9 base editor fusion proteins. Suitable protein tags provided herein include, but are not limited to, biotin carboxylase carrier protein (BCCP) tags, myc-tags, calmodulin-tags, FLAG-tags, hemagglutinin (HA)-tags, polyhistidine tags, also referred to as histidine tags or His-tags, maltose binding protein (MBP)-tags, nus-tags, glutathione-S-transferase (GST)-tags, green fluorescent protein (GFP)-tags, thioredoxin-tags, S-tags, Softags (e.g., Softag 1, Softag 3), strep-tags, biotin ligase tags, FLASH tags, V5 tags, and SBP-tags. Additional suitable sequences will be apparent to those of skill in the art. In some embodiments, the GeoCas9 base editor fusion protein comprises one or more His tags.

[0161] In some embodiments, the deaminase domain and the GeoCas9 are fused to each other via a linker. Various linker lengths and flexibilities between the deaminase domain (e.g., AID) and the Cas9 domain can be employed (e.g., ranging from very flexible linkers of the form $(GGGS)_n$ (SEQ ID NO: 9) $(GGGGS)_n$ (SEQ ID NO: 10), (GGS), (SEQ ID NO: 13), and $(G)_n$ (SEQ ID NO: 11) to more rigid linkers of the form $(EAAAK)_n$ (SEQ ID NO: 12), $(SGGS)_n$ (SEQ ID NO: 14), SGSETPGTSESATPES (SEQ ID NO: 15) (see, e.g., Guilinger J P, Thompson D B, Liu D R. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32 (6): 577-82; the entire contents are incorporated herein by reference) and (XP)n) (SEQ ID NO: 18) in order to achieve the optimal length for deaminase activity for the specific application. In some embodiments, the linker comprises a $(GGS)_n$ (SEQ ID NO: 13) motif, wherein n is 1, 3, or 7. In some embodiments, the linker comprises a (an SGSETPGTSESATPES (SEQ ID NO: 15) motif.

[0162] Some exemplary suitable nucleic-acid editing domains, e.g., deaminases and deaminase domains that can be fused to GeoCas9 according to aspects of this disclosure are provided below. It should be understood that, in some embodiments, the active domain of the respective sequence can be used, e.g., the domain without a localizing signal (nuclear localization sequence, without nuclear export signal, cytoplasmic localizing signal). Examples of cytidine deaminase amino acid sequences with an NLS sequence (single underline) and/or nuclear export signals (double underline) are shown, as follows:

Human AID:

(SEQ ID NO: 25)

MDSLLMNRRKFLYQFKNVRWAKGRRETYLCYVVKRRDSATSFSLDFGYLRNKNGC

HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTAR

LYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHEN

SVRLSROLRRILLPLYEVDDLRDAFRTLGL

(underline: nuclear localization sequence; double underline: nuclear export signal)

-continued

Mouse AID:
(SEQ ID NO: 26)
MDSLLMKQKKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSCSLDFGHLRNKSGC

HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVAEFLRWNPNLSLRIFTAR

LYFCEDRKAEPEGLRRLHRAGVQIGIMTFKDYFYCWNTFVENRERTFKAWEGLHEN

SVRLTRQLRRILLPLYEVDDLRDAFRMLGF
(underline: nuclear localization sequence; double underline: nuclear export signal)

Dog AID:
(SEQ ID NO: 27)
MDSLLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGHLRNKSGC

HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFAAR

LYFCEDRKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENREKTFKAWEGLHEN

SVRLSRQLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence; double underline: nuclear export signal)

Bovine AID:
(SEQ ID NO: 28)
MDSLLKKQRQFLYQFKNVRWAKGRHETYLCYVVKRRDSPTSFSLDFGHLRNKAGC

HVELLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGYPNLSLRIFTAR

LYFCDKERKAEPEGLRRLHRAGVQIAIMTFKDYFYCWNTFVENHERTFKAWEGLHE

NSVRLSRQLRRILLPLYEVDDLRDAFRTLGL
(underline: nuclear localization sequence; double underline: nuclear export signal)

Rat AID:
(SEQ ID NO: 44)
MAVGSKPKAALVGPHWERERIWCFLCSTGLGTQQTGQTSRWLRPAATQDPVSPPRS

LLMKQRKFLYHFKNVRWAKGRHETYLCYVVKRRDSATSFSLDFGYLRNKSGCHVE

LLFLRYISDWDLDPGRCYRVTWFTSWSPCYDCARHVADFLRGNPNLSLRIFTARLTG

WGALPAGLMSPARPSDYFYCWNTFVENHERTFKAWEGLHENSVRLSRRLRRILLPL

YEVDDLRDAFRTLGL
(underline: nuclear localization sequence; double underline: nuclear export signal)

Rhesus macaque APOBEC-3G:
(SEQ ID NO: 31)
MVEPMDPRTFVSNFNNRPILSGLNTVWLCCEVKTKDPSGPPLDAKIFQGKVYSKAKY

*HPEMRFLRWFHKWRQLHHDQEYKVTWYVSWSPCTRC*ANSVAEFEAKDPKVEEE1FVA

RLYYFWKPDYQQALRILCQKRGGPHATMKIMNYNEFQDCWNKFVDGRGKPFKPRN

NLPKHYTLLQATLGELLRHLMDPGTFTSNFNNKPWVSGQHETYLCYKVERLHNDT

WNPENQHRGFERNQAPNIHGFPKGR*HAELCFLDLIPFWKLDGQQYRVTCFTSWSPCFS*

CAQEMAKFISNNEHVSLCIFAARIYDDQGRYQEGLRALHRDGAKIAMMNYSEFEYC

WDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI
(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

-continued

Chimpanzee APOBEC-3G:

(SEQ ID NO: 32)

MKPHFRNPVERMYQDTFSDNFYNRPILSHRNTVWLCYEVKTKGPSRPPLDAKIFRGQ

VYSKLKA*HPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDVAEFEAEDPKV

TLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRE

LFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTSNFNNELWVRGRHETYLCYEVERL

HNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLDLHQDYRVTCFTS*

*WSPCFSC*AQEMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLAKAGAKISIMTYSE

FKHCWDTFVDHQGCPFQPWDGLEEHSQALSGRLRAILQNQGN
(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Green monkey APOBEC-3G:

(SEQ ID NO: 33)

MNPQIRNMVEOMEPDIFVYYFNNRPILSGRNTVWLCYEVKTKDPSGPPLDANIFQGK

EYPEAKD*HPEMKFLHWFRKWRQLHRDQEYEVTWYVSWSPCTRC*ANSVAEFEAEDPKV

TLTIFVARLYYFWKPDYQQALRILCQERGGPHATMKIMNYNEFQHCWNEFVDGQG

KPFKPRKNLPKHYTLLHATLGELLRHVMDPGTFTSNFNNKPWVSGQRETYLCYKVE

RSHNDTWVLLNQHRGFLRNQAPDRHGFPKGR*HAELCFLDLIPFWKLDDOQYRVTCFT*

*SWSPCFSC*AQKMAKFISNNKHVSLCIFAARIYDDQGRCQEGLRTLHRDGAKIAVMNY

SEFEYCWDTFVDRQGRPFQPWDGLDEHSQALSGRLRAI
(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3G:

(SEQ ID NO: 34)

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPPLDAKIFRGO

VYSELKY*HPEMRFFHWFSKWRKLHRDQEYEVTWYISWSPCTKC*TRDMATFLAEDPKV

TLTIFVARLYYFWDPDYQEALRSLCQKRDGPRATMKIMNYDEFQHCWSKFVYSQRE

LFEPWNNLPKYYILLHIMLGEILRHSMDPPTFTFNFNNEPWVRGRHETYLCYEVERM

HNDTWVLLNQRRGFLCNQAPHKHGFLEGR*HAELCFLDVIPFWKLDLDODYRVTCFTS*

*WSPCFSC*AQEMAKFISKNKHVSLCIFTARIYDDQGRCQEGLRTLAEAGAKISIMTYSE

FKHCWDTFVDHQGCPFQPWDGLDEHSQDLSGRLRAILQNQEN
(italic: nucleic acid editing domain; underline: cytoplasmic localization signal)

Human APOBEC-3F:

(SEQ ID NO: 35)

MKPHFRNTVERMYRDTFSYNFYNRPILSRRNTVWLCYEVKTKGPSRPRLDAKIFRGQ

VYSQPEH*HAEMCFLSWFCGNQLPAYKCFQITWFVSWTPCPDC*VAKEAEEEAEHPNVYE

TISAARLYYYWERDYRRALCRLSQAGARVKIMDDEEFAYCWENFVYSEGQPFMPW

YKFDDNYAFLHRTLKEILRNPMEAMYPHIFYFHFKNLRKAYGRNESWLCFTMEVVK

HHSPNSWKRGNERNQNDPEYH*CHAERCFLSWFCDDILSPNTNYEVTWYTSWSPCPEC*A

GEVAEFLARHSNVNLTIFTARLYYFWDTDYQEGLRSLSQEGASVEIMGYKDFKYCW

ENFVYNDDEPFKPWKGLKYNFLFLDSKLQEILE
(italic: nucleic acid editing domain)

J. UGI Domains

[0163] In some aspects of the disclosure, the GeoCas9 base editor fusion proteins may comprise one or more uracil glycosylase inhibitor (UGI) domains. In some embodiments, the GeoCas9 domain (e.g., a nuclease active GeoCas9 domain, a nuclease inactive dGeoCas9 domain, or a GeoCas9 nickase) may be fused to one or more UGI domains either directly or via a linker. Without wishing to be bound by any particular theory, cellular DNA-repair response to the presence of U: G heteroduplex DNA may be responsible for

the decrease in nucleobase editing efficiency in cells. For example, uracil DNA glycosylase (UDG) catalyzes removal of U from DNA in cells, which may initiate base excision repair, with reversion of the U: G pair to a C: G pair as the most common outcome. Uracil DNA Glycosylase Inhibitor (UGI) inhibits human UDG activity. Thus, this disclosure contemplates a GeoCas9 base editor fusion protein comprising GeoCas9 domain, a nucleic acid effector domain (e.g., a cytidine or adenosine deaminase), and one or more UGI domains. Each of the domains may be joined through one or more linkers. It should be understood that the use of a UGI domain may increase the editing efficiency of a nucleic acid editing domain that is capable of catalyzing a C to U change. For example, GeoCas9 base editor fusion proteins comprising a UGI domain may be more efficient in deaminating C residues. In some embodiments, the GeoCas9 base editor fusion protein comprises the structure:

[0164] [NH^2]-[deaminase domain]-[GeoCas9]-[UGI domain]-[COOH];

[0165] [NH^2]-[nucleic acid effector domain]-[UGI domain]-[GeoCas9]-[COOH];

[0166] [NH^2]-[UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[COOH];

[0167] [NH^2]-[UGI domain]-[GeoCas9]-[nucleic acid effector domain]-[COOH];

[0168] [NH^2]-[GeoCas9]-[nucleic acid effector domain]-[UGI domain]-[COOH];

[0169] [NH^2]-[GeoCas9]-[UGI domain]-[nucleic acid effector domain]-[COOH];

[0170] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[COOH];

[0171] [NH^2]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[COOH];

[0172] [NH^2]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[COOH];

[0173] [NH^2]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[nucleic acid effector domain]-[COOH];

[0174] [NH^2]-[GeoCas9]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[COOH];

[0175] [NH^2]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[COOH];

[0176] [NH^2]-[NLS]-[nucleic acid effector domain]-[GeoCas9]-[UGI domain]-[COOH];

[0177] [NH^2]-[NLS]-[nucleic acid effector domain]-[UGI domain]-[GeoCas9]-[COOH];

[0178] [NH^2]-[NLS]-[UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[COOH];

[0179] [NH^2]-[NLS]-[UGI domain]-[GeoCas9 domain]-[deaminase]-[COOH];

[0180] [NH^2]-[NLS]-[GeoCas9]-[nucleic acid effector domain]-[UGI domain]-[COOH];

[0181] [NH^2]-[NLS]-[GeoCas9]-[UGI domain]-[nucleic acid effector domain]-[COOH];

[0182] [NH^2]-[NLS]-[nucleic acid effector domain]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[COOH];

[0183] [NH^2]-[NLS]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[COOH];

[0184] [NH^2]-[NLS]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[COOH];

[0185] [NH^2]-[NLS]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[nucleic acid effector domain]-[COOH];

[0186] [NH^2]-[NLS]-[GeoCas9]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[COOH];

[0187] [NH^2]-[NLS]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[COOH];

[0188] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[UGI domain]-[NLS]-[COOH];

[0189] [NH^2]-[nucleic acid effector domain]-[UGI domain]-[GeoCas9]-[NLS]-[COOH];

[0190] [NH^2]-[UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[NLS]-[COOH];

[0191] [NH^2]-[UGI domain]-[GeoCas9]-[nucleic acid effector domain]-[NLS]-[COOH];

[0192] [NH^2]-[GeoCas9]-[nucleic acid effector domain]-[UGI domain]-[NLS]-[COOH];

[0193] [NH^2]-[GeoCas9]-[UGI domain]-[nucleic acid effector domain]-[NLS]-[COOH];

[0194] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[NLS]-[COOH];

[0195] [NH^2]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[NLS]-[COOH];

[0196] [NH^2]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[GeoCas9]-[NLS]-[COOH];

[0197] [NH^2]-[first UGI domain]-[second UGI domain]-[GeoCas9]-[nucleic acid effector domain]-[NLS]-[COOH];

[0198] [NH^2]-[GeoCas9]-[nucleic acid effector domain]-[first UGI domain]-[second UGI domain]-[NLS]-[COOH]; or

[0199] [NH^2]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[nucleic acid effector domain]-[NLS]-[COOH].

[0200] In various embodiments, the nucleic acid effector domain in these constructs can be a cytidine deaminase. In other embodiment, the nucleic acid effector domain in these constructs can be an adenosine deaminase. In still other embodiments, the nucleic acid effector domain can be a recombinase domain. In yet other embodiments, the nucleic acid effector domain can be a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain.

[0201] In some embodiments, a UGI domain comprises a wild-type UGI or a UGI as set forth in SEQ ID NO: 60. In some embodiments, the UGI proteins provided herein include fragments of UGI and proteins homologous to a UGI or a UGI fragment. For example, in some embodiments, a UGI domain comprises a fragment of the amino acid sequence set forth in SEQ ID NO: 60. In some embodiments, a UGI fragment comprises an amino acid sequence that comprises at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% of the amino acid sequence as set forth in SEQ ID NO: 60. In some embodiments, a UGI comprises an

amino acid sequence homologous to the amino acid sequence set forth in SEQ ID NO: 60 or an amino acid sequence homologous to a fragment of the amino acid sequence set forth in SEQ ID NO: 60. In some embodiments, proteins comprising UGI or fragments of UGI or homologs of UGI or UGI fragments are referred to as "UGI variants." A UGI variant shares homology to UGI, or a fragment thereof.

[0202] For example a UGI variant is at least 70% identical, at least 75% identical, at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% identical to a wild type UGI or a UGI as set forth in SEQ ID NO: 60. In some embodiments, the UGI variant comprises a fragment of UGI, such that the fragment is at least 70% identical, at least 80% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical, at least 99.5% identical, or at least 99.9% to the corresponding fragment of wild-type UGI or a UGI as set forth in SEQ ID NO: 60. In some embodiments, the UGI comprises the following amino acid sequence:

```
>sp|P14739|UNGI_BPPB2 Uracil-DNA glycosylase
inhibitor
                                    (SEQ ID NO: 60)
MTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVH

TAYDESTDENVMLLTSDAPEYKPWALVIQDSNGENKIKML
```

[0203] Suitable UGI protein and nucleotide sequences are provided herein and additional suitable UGI sequences are known to those in the art, and include, for example, those published in Wang et al., Uracil-DNA glycosylase inhibitor gene of bacteriophage PBS2 encodes a binding protein specific for uracil-DNA glycosylase. J. Biol. Chem. 264: 1163-1171 (1989); Lundquist et al., Site-directed mutagenesis and characterization of uracil-DNA glycosylase inhibitor protein. Role of specific carboxylic amino acids in complex formation with *Escherichia coli* uracil-DNA glycosylase. J. Biol. Chem. 272:21408-21419 (1997); Ravishankar et al., X-ray analysis of a complex of *Escherichia coli* uracil DNA glycosylase (EcUDG) with a proteinaceous inhibitor. The structure elucidation of a prokaryotic UDG. Nucleic Acids Res. 26:4880-4887 (1998); and Putnam et al., Protein mimicry of DNA from crystal structures of the uracil-DNA glycosylase inhibitor protein and its complex with *Escherichia coli* uracil-DNA glycosylase. J. Mol. Biol. 287:331-346 (1999), the entire contents of each are incorporated herein by reference.

[0204] It should be appreciated that additional proteins may be uracil glycosylase inhibitors. For example, other proteins that are capable of inhibiting (e.g., sterically blocking) a uracil-DNA glycosylase base-excision repair enzyme are within the scope of this disclosure. Additionally, any proteins that block or inhibit base-excision repair as also within the scope of this disclosure. In some embodiments, the GeoCas9 base editor fusion proteins described herein comprise one UGI domain. In some emobidments, the GeoCas9 base editor fusion proteins described herein comprise more than one UGI domain. In some embodiments, the GeoCas9 base editor fusion proteins described herein comprise two UGI domains. In some embodiments, the GeoCas9 base editor fusion proteins described herein comprise more than two UGI domains. In some embodiments, a protein that binds DNA is used. In another embodiment, a substitute for UGI is used. In some embodiments, a uracil glycosylase inhibitor is a protein that binds single-stranded DNA.

[0205] For example, a uracil glycosylase inhibitor may be a *Erwinia* tasmaniensis single-stranded binding protein. In some embodiments, the single-stranded binding protein comprises the amino acid sequence (SEQ ID NO: 61). In some embodiments, a uracil glycosylase inhibitor is a protein that binds uracil. In some embodiments, a uracil glycosylase inhibitor is a protein that binds uracil in DNA. In some embodiments, a uracil glycosylase inhibitor is a catalytically inactive uracil DNA-glycosylase protein. In some embodiments, a uracil glycosylase inhibitor is a catalytically inactive uracil DNA-glycosylase protein that does not excise uracil from the DNA. For example, a uracil glycosylase inhibitor is a UdgX. In some embodiments, the UdgX comprises the amino acid sequence (SEQ ID NO: 62). As another example, a uracil glycosylase inhibitor is a catalytically inactive UDG. In some embodiments, a catalytically inactive UDG comprises the amino acid sequence (SEQ ID NO: 63).

[0206] It should be appreciated that other uracil glycosylase inhibitors would be apparent to the skilled artisan and are within the scope of this disclosure. In some embodiments, a uracil glycosylase inhibitor is a protein that is homologous to any one of SEQ ID NOs: 60-74. In some embodiments, a uracil glycosylase inhibitor is a protein that is at least 50% identical, at least 55% identical at least 60% identical, at least 65% identical, at least 70% identical, at least 75% identical, at least 80% identical at least 85% identical, at least 90% identical, at least 95% identical, at least 96% identical, at least 98% identical, at least 99% identical, or at least 99.5% identical to any one of SEQ ID NOs: 60-74. Additional suitable UGI sequences are as follows.

Erwinia tasmaniensis SSB
(themostable single-stranded DNA binding protein)
(SEQ ID NO: 61)
MASRGVNKVILVGNLGQDPEVRYMPNGGAVANITLATSESWRDKQTGET

KEKTEWHRVVLFGKLAEVAGEYLRKGSQVYIEGALQTRKWTDQAGVEKY

TTEVVVNVGGTMQMLGGRSQGGGASAGGQNGGSNNGWGQPQQPQGGNQF

SGGAQQQARPQQQPQQNNAPANNEPPIDFDDDIP

UdgX (binds to Uracil in DNA but does not excise)
(SEQ ID NO: 62)
MAGAQDFVPHTADLAELAAAAGECRGCGLYRDATQAVFGAGGRSARIMM

IGEQPGDKEDLAGLPFVGPAGRLLDRALEAADIDRDALYVTNAVKHFKF

TRAAGGKRRIHKTPSRTEVVACRPWLIAEMTSVEPDVVVLLGATAAKAL

LGNDFRVTQHRGEVLHVDDVPGDPALVATVHPSSLLRGPKEERESAFAG

LVDDLRVAADVRP

UDG (catalytically inactive human UDG, binds to Uracil in DNA but does not excise)
(SEQ ID NO: 63)
MIGQKTLYSFFSPSPARKRHAPSPEPAVQGTGVAGVPEESGDAAAIPAK

KAPAGQEEPGTPPSSPLSAEQLDRIQRNKAAALLRLAARNVPVGFGESW

KKHLSGEFGKPYFIKLMGFVAEERKHYTVYPPPHQVFTWTQMCDIKDVK

-continued

VVILGQEPYHGPNQAHGLCFSVQRPVPPPPSLENIYKELSTDIEDFVHP

GHGDLSGWAKQGVLLLNAVLTVRAHQANSHKERGWEQFTDAVVSWLNQN

SNGLVFLLWGSYAQKKGSAIDRKRHHVLQTAHPSPLSVYRGFFGCRHFS

KTNELLQKSGKKPIDWKEL

[0207] Additional single-stranded DNA binding proteins that can be used as a UGI are shown below. It should be appreciated that other single-stranded binding proteins may be used as a UGI, for example those described in Dickey T H, Altschuler S E, Wuttke D S. Single-stranded DNA-binding proteins: multiple domains for multiple functions. *Structure.* 2013 Jul. 2; 21 (7): 1074-84. doi: 10.1016/j.str.2013.05.013. Review.; Marceau A H. Functions of single-strand DNA-binding proteins in DNA replication, recombination, and repair. *Methods Mol Biol.* 2012; 922:1-21. doi: 10.1007/978-1-62703-032-8_1.; Mijakovic, Ivan, et al.; Bacterial single-stranded DNA-binding proteins are phosphorylated on tyrosine. *Nucleic Acids Res* 2006; 34 (5): 1588-1596. doi: 10.1093/nar/gkj514; Mumtsidu E, Makhov A M, Konarev P V, Svergun D I, Griffith J D, Tucker P A. Structural features of the single-stranded DNA-binding protein of Epstein-Barrvirus. *J Struct Biol.* 2008 February; 161 (2): 172-87. Epub 2007 Nov. 1; Nowak M, Olszewski M, Śpibida M, Kur J. Characterization of single-stranded DNA-binding proteins from the psychrophilic bacteria *Desulfotalea psychrophila, Flavobacterium psychrophilum, Psychrobacter arcticus, Psychrobactercryohalolentis, Psychromonas ingrahamii, Psychroflexus torquis*, and *Photobacterium profundum. BMC Microbiol.* 2014 Apr. 14; 14:91. doi: 10.1186/1471-2180-14-91; Tone T, Takeuchi A, Makino O. Single-stranded DNA binding protein Gp5 of *Bacillus subtilis* phage Φ29 is required for viral DNA replication in growth-temperature dependent fashion. *Biosci Biotechnol Biochem.* 2012; 76 (12): 2351-3. Epub 2012 Dec. 7; Wold. REPLICATION PROTEIN A: A Heterotrimeric, Single-Stranded DNA-Binding Protein Required for Eukaryotic DNA Metabolism. *Annual Review of Biochem.* 1997; 66:61-92. doi: 10.1146/annurev.biochem.66.1.61; Wu Y, Lu J, Kang T. Human single-stranded DNA binding proteins: guardians of genome stability. *Acta Biochim Biophys Sin* (Shanghai). 2016 July; 48 (7): 671-7. doi: 10.1093/abbs/gmw044. Epub 2016 May 23. Review; the entire contents of each are hereby incorporated by reference.

mtSSB-SSBP1 single stranded DNA binding protein 1
[*Homo sapiens* (human)] (UniProtKB: Q04837; NP_001243439.1)
(SEQ ID NO: 64)

MFRRPVLQVLRQFVRHESETTTSLVLERSLNRVHLLGRVGQDPVLRQVEG

KNPVTIFSLATNEMWRSGDSEVYQLGDVSQKTTWHRISVFRPGLRDVAYQYVKKGS

RIYLEGKIDYGEYMDKNNVRRQATTIIADNIIFLSDQTKEKE

Single-stranded DNA-binding protein 3 isoform A [*Mus musculus*]
(UniProtKB-Q9D032-1; NCBI Ref: NP_076161.2)
(SEQ ID NO: 65)

MFAKGKGSAVPSDGQAREKLALYVYEYLLHVGAQKSAQTFLSEIRWEKNI

TLGEPPGFLHSWWCVFWDLYCAAPERRDTCEHSSEAKAFHDYSAAAAPSPVLGNIPP

NDGMPGGPIPPGFFQGPPGSQPSPHAQPPPHNPSSMMGPHSQPFMSPRYAGGPRPPIR

MGNQPPGGVPGTQPLLPNSMDPTRQQGHPNMGGSMQRMNPPRGMGPMGPGPQNY

GSGMRPPPNSLGPAMPGINMGPGAGRPWPNPNSANSIPYSSSSPGTYVGPPGGGGPP

GTPIMPSPADSTNSSDNIYTMINPVPPGGSRSNFPMGPGSDGPMGGMGGMEPHHMN

GSLGSGDIDGLPKNSPNNISGISNPPGTPRDDGELGGNFLHSFQNDNYSPSMTMSV

RPA 1-Replication protein A 70 kDa DNA-binding subunit
(UniProtKB: P27694; NCBI Ref: NM_002945.3)
(SEQ ID NO: 66)

MVGQLSEGAIAAIMQKGDTNIKPILQVINIRPITTGNSPPRYRLLMSDGLNT

LSSFMLATQLNPLVEEEQLSSNCVCQIHRFIVNTLKDGRRVVILMELEVLKSAEAVGV

KIGNPVPYNEGLGQPQVAPPAPAASPAASSRPQPQNGSSGMGSTVSKAYGASKTFGK

AAGPSLSHTSGGTQSKVVPIASLTPYQSKWTICARVTNKSQIRTWSNSRGEGKLFSLE

LVDESGEIRATAFNEQVDKFFPLIEVNKVYYFSKGTLKIANKQFTAVKNDYEMTFNN

ETSVMPCEDDHHLPTVQFDFTGIDDLENKSKDSLVDIIGICKSYEDATKITVRSNNRE

VAKRNIYLMDTSGKVVTATLWGEDADKFDGSRQPVLAIKGARVSDFGGRSLSVLSS

-continued

STIIANPDIPEAYKLRGWFDAEGQALDGVSISDLKSGGVGGSNTNWKTLYEVKSENL

GQGDKPDYFSSVATVVYLRKENCMYQACPTQDCNKKVIDQQNGLYRCEKCDTEFP

NFKYRMILSVNIADFQENQWVTCFQESAEAILGQNAAYLGELKDKNEQAFEEVFQN

ANFRSFIFRVRVKVETYNDESRIKATVMDVKPVDYREYGRRLVMSIRRSALM

RPA 2-Replication protein A 32 kDa subunit (UniProtKB: P15927; NCBI Ref: NM_002946)

(SEQ ID NO: 67)

MWNSGFESYGSSSYGGAGGYTQSPGGFGSPAPSQAEKKSRARAQHIVPCTI

SQLLSATLVDEVFRIGNVEISQVTIVGIIRHAEKAPTNIVYKIDDMTAAPMDVRQWVD

TDDTSSENTVVPPETYVKVAGHLRSFQNKKSLVAFKIMPLEDMNEFTTHILEVINAH

MVLSKANSQPSAGRAPISNPGMSEAGNFGGNSFMPANGLTVAQNQVLNLIKACPRP

EGLNFQDLKNQLKHMSVSSIKQAVDFLSNEGHIYSTVDDDHFKSTDAE

RPA 3-Replication protein A 14 kDa subunit (UniProtKB: P35244; NCBI Ref: NM_002947.4)

(SEQ ID NO: 68)

MVDMMDLPRSRINAGMLAQFIDKPVCFVGRLEKIHPTGKMFILSDGEGKN

GTIELMEPLDEEISGIVEVVGRVTAKATILCTSYVQFKEDSHPFDLGLYNEAVKIIHDF

PQFYPLGIVQHD

ssbA-single-stranded DNA-binding protein [*Bacillus subtilis* subsp. *subtilis* str. 168] (UniProtKB: P37455; NCBI Ref:)

(SEQ ID NO: 69)

MLNRVVLVGR LTKDPELRYT PNGAAVATFT LAVNRTFTNQ

SGEREADFINCVTWRRQAEN VANFLKKGSL AGVDGRLQTR NYENQQGQRV

FVTEVQAESVQFLEPKNGGG SGSGGYNEGN SGGGQYFGGG QNDNPFGGNQ

NNQRRNQGNSFNDDPFANDG KPIDISDDDLPF

Single-stranded DNA-binding protein 2 [*Streptomyces coelicolor* A3(2)] (UniProtKB: Q9X8U3; NCBI Ref: NP_628093.1)

(SEQ ID NO: 70)

MAGETVITVVGNLVDDPELRFTPSGAAVAKFRVASTPRTFDRQTNEWKDG

ESLFLTCSVWRQAAENVAESLQRGMRVIVQGRLKQRSYEDREGVKRTVYELDVDEV

GASLRSATAKVTKTSGQGRGGQGGYGGGGGGQGGGGWGGGPGGGQQGGGAPAD

DPWATGGAPAGGQQGGGGQGGGGWGGGSGGGGGYSDEPPF

Single-stranded DNA-binding protein [*Streptococcus pneumoniae* R6] (UniProtKB: P66855; NCBI Ref: NP_358988.1)

(SEQ ID NO: 71)

MINNVVLVGRMTRDAELRYTPSNVAVATFTLAVNRTFKSQNGEREADFIN

VVMWRQQAENLANWAKKGSLIGVTGRIQTRSYDNQQGQRVYVTEVVAENFQMLE

SRSVREGHTGGAYSAPTANYSAPTNSVPDFSRNENPFGATNPLDISDDDLPF

Single-stranded DNA-binding protein [Human alphaherpesvirus 1] (UniProtKB: P04296; NCBI Ref: YP_009137104.1)

(SEQ ID NO: 72)

METKPKTATTIKVPPGPLGYVYARACPSEGIELLALLSARSGDSDVAVAPL

VVGLTVESGFEANVAVVVGSRTTGLGGTAVSLKLTPSHYSSSVYVFHGGRHLDPST

QAPNLTRLCERARRHFGFSDYTPRPGDLKHETTGEALCERLGLDPDRALLYLVVTEG

FKEAVCINNTFLHLGGSDKVTIGGAEVHRIPVYPLQLFMPDFSRVIAEPFNANHRSIGE

NFTYPLPFFNRPLNRLLFEAVVGPAAVALRCRNVDAVARAAAHLAFDENHEGAALP

ADITFTAFEASQGKTPRGGRDGGGKGPAGGFEQRLASVMAGDAALALESIVSMAVF

DEPPTDISAWPLFEGQDTAAARANAVGAYLARAAGLVGAMVFSTNSALHLTEVDD

-continued

AGPADPKDHSKPSFYRFFLVPGTHVAANPQVDREGHVVPGFEGRPTAPLVGGTQEF

AGEHLAMLCGFSPALLAKMLFYLERCDGGVIVGRQEMDVFRYVADSNQTDVPCNL

CTFDTRHACVHTTLMRLRARHPKFASAARGAIGVFGTMNSMYSDCDVLGNYAAFS

ALKRADGSETARTIMQETYRAATERVMAELETLQYVDQAVPTAMGRLETIITNREAL

HTVVNNVRQVVDREVEQLMRNLVEGRNFKFRDGLGEANHAMSLTLDPYACGPCPL

LQLLGRRSNLAVYQDLALSQCHGVFAGQSVEGRNFRNQFQPVLRRRVMDMFNNGF

LSAKTLTVALSEGAAICAPSLTAGQTAPAESSFEGDVARVTLGFPKELRVKSRVLFAG

ASANASEAAKARVASLQSAYQKPDKRVDILLGPLGFLLKQFHAAIFPNGKPPGSNQP

NPQWFWTALQRNQLPARLLSREDIETIAFIKKFSLDYGAINFINLAPNNVSELAMYYM

ANQILRYCDHSTYFINTLTAIIAGSRRPPSVQAAAAWSAQGGAGLEAGARALMDAV

DAHPGAWTSMFASCNLLRPVMAARPMVVLGLSISKYYGMAGNDRVFQAGNWASL

MGGKNACPLLIFDRTRKFVLACPRAGFVCAASSLGGGAHESSLCEQLRGIISEGGAA

VASSVFVATVKSLGPRTQQLQIEDWLALLEDEYLSEEMMELTARALERGNGEWSTD

AALEVAHEAEALVSQLGNAGEVFNFGDFGCEDDNATPFGGPGAPGPAFAGRKRAFH

GDDPFGEGPPDKKGDLTLDML

Single-stranded DNA-binding protein from *Bacillus* virus phi29
(UniProtKB: Q38504.1; NCBI Ref: YP_002004532.1)
(SEQ ID NO: 73)

MENTNIVKATFDTETLEGQIKIFNAQTGGGQSFKNLPDGTIIEANAIAQYKQ

VSDTYGDAKEETVTTIFAADGSLYSAISKTVAEAASDLIDLVTRHKLETFKVKVVQG

TSSKGNVFFSLQLSL

Single stranded DNA binding protein [*Burkholderia* virus DC1]
(UniProtKB: I6NRL7; NCBI Ref: YP_006589943.1)
(SEQ ID NO: 74)

MASVNKVILVGNLGADPETRYLPSGDAISNIRLATTDRYKDKASGEMKEST

EWHRVSFFGRLAEIVDEYLRKGAPVYIEGRIRTRKWQDNAGQDRYTTEIVAEKMQM

LGDRRDGGERQQRAPQQQQQRTQRNGYADATGRAQPSQRPAAGGGFDEMDDDIPF

[0208] Suitable UGI protein and nucleotide sequences are provided herein and additional suitable UGI sequences are known to those in the art, and include, for example, those published in Wang et al., Uracil-DNA glycosylase inhibitor gene of bacteriophage PBS2 encodes a binding protein specific for uracil-DNA glycosylase. *J. Biol. Chem.* 264: 1163-1171 (1989); Lundquist et al., Site-directed mutagenesis and characterization of uracil-DNA glycosylase inhibitor protein. Role of specific carboxylic amino acids in complex formation with *Escherichia coli* uracil-DNA glycosylase. *J. Biol. Chem.* 272:21408-21419 (1997); Ravishankar et al., X-ray analysis of a complex of *Escherichia coli* uracil DNA glycosylase (EcUDG) with a proteinaceous inhibitor. The structure elucidation of a prokaryotic UDG. *Nucleic Acids Res.* 26:4880-4887 (1998); and Putnam et al., Protein mimicry of DNA from crystal structures of the uracil-DNA glycosylase inhibitor protein and its complex with *Escherichia coli* uracil-DNA glycosylase. *J. Mol. Biol.* 287:331-346 (1999), the entire contents of which are incorporated herein by reference. In some embodiments, the optional linker comprises a (GGS)n motif, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 19, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In some embodiments, the optional linker comprises a (GGS), motif, wherein n is 1, 3, or 7. In some embodiments, the optional linker comprises the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 15), which is also referred to as the XTEN linker in the Examples.

K. GeoCas9 Base Editor Fusion Proteins

[0209] In various aspects, the disclosure provide GeoCas9 base editors which are fusion proteins comprising two or more functional domains, optionally joined by one or linkers. These proteins may also be referred to as GeoCas9 base editor fusion proteins, or in some cases, fusion proteins. The functional domains can include a GeoCas9 domain (e.g., a nuclease active GeoCas9 protein, a nuclease-inactive dGeoCas9 protein, or a GeoCas9 nickase protein) and one or more nucleic acid effector domains, such as, but not limited to, a cytidine deaminase, an adenosine deaminase, a nuclease, a nickase, a recombinase, a methyltransferase, a methylase, an acetylase, an acetyltransferase, a transcriptional activator, or a transcriptional repressor domain. Any of these functional domains may be joined by one or more linkers. In addition, the GeoCas9 base editors may comprise additional sequences, including, but not limited to, one or more nuclear localization sequences, UGI domains (one or more) and

other sequence tags. The one or more UGI domains may also be joined by one or more linker sequences.

[0210] The GeoCas9 base editors are not particularly limited in the arrangement of its functional domains, i.e., the linear order of functional domains from the amino-terminus to the carboxy-terminus of the GeoCas9 base editor fusion proteins.

[0211] In some embodiments, the general architecture of exemplary GeoCas9 fusion proteins with a nucleic acid effector domain (e.g., a cytidine deaminase, adenosine deaminase, recombinase, methylase, methyltransferase, etc.) comprises the structure:

[0212] [NH_2]-[NLS]-[nucleic acid effector domain]-[GeoCas9]-[COOH];

[0213] [NH_2]-[NLS]-[GeoCas9]-[nucleic acid effector domain]-[COOH];

[0214] [NH_2]-[nucleic acid effector domain]-[GeoCas9]-[NLS]-[COOH]; or

[0215] [NH_2]-[GeoCas9]-[nucleic acid effector domain]-[NLS]-[COOH];

[0216] wherein NLS is a nuclear localization sequence, NH_2 is the N-terminus of the GeoCas9 base editor fusion protein, COOH is the C-terminus of the GeoCas9 base editor fusion protein, and wherein each instance of "-" comprises an optional linker, for example any of the linkers provided herein. Nuclear localization sequences are known in the art and would be apparent to the skilled artisan. For example, NLS sequences are described in Plank et al., PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In some embodiments, a NLS comprises the amino acid sequence PKKKRKV (SEQ ID NO: 75) or MDSLLMNRRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 76).

[0217] In some embodiments, a linker is inserted between the GeoCas9 and the deaminase. In some embodiments, the NLS is located C-terminal of the GeoCas9 domain. In some embodiments, the NLS is located N-terminal of the GeoCas9 domain. In some embodiments, the NLS is located between the deaminase and the GeoCas9 domain. In some embodiments, the NLS is located N-terminal of the deaminase domain. In some embodiments, the NLS is located C-terminal of the deaminase domain.

[0218] Some aspects of this disclosure provide fusion proteins comprising (i) a GeoCas9, such as a nuclease active GeoCas9, a nuclease inactive dGeoCas9, or a GeoCas9 nickase; and (ii) an effector domain, such as a cytidine deaminase domain. In some embodiments, the GeoCas9 (e.g., GeoCas9, dGeoCas9, or GeoCas9n), comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to the amino acid sequence of a GeoCas9 as provided by any one of SEQ ID NOs: 2, 3, 5-8. In some embodiments, the GeoCas9 comprises one or more mutations that inactivate or alter (e.g., yield a nickase) the nuclease activity of GeoCas9. Mutations that render one or more nuclease domains of Cas9 inactive are well-known in the art. For example, the DNA cleavage domain of GeoCas9 is known to include two subdomains, the HNH nuclease subdomain and the RuvC1 subdomain. The HNH subdomain cleaves the strand complementary to the gRNA, whereas the RuvC1 subdomain cleaves the non-complementary strand. Mutations within these subdomains can silence the nuclease activity of GeoCas9. For example, the mutations D8A and H582A could inactivate the nuclease activity of GeoCas9. In some embodiments, the dGeoCas9 of this disclosure comprises a D8A mutation of the amino acid sequence provided in SEQ ID NO: 5, or a corresponding mutation in any of the amino acid sequences provided in SEQ ID NOs: 6-8.

[0219] In some embodiments, the dGeoCas9 of this disclosure comprises a H582A mutation of the amino acid sequence provided in SEQ ID NO: 5, or a corresponding mutation in any of the amino acid sequences provided in SEQ ID NOs: 6-8. In some embodiments, the dGeoCas9 of this disclosure comprises both D8A and H582A mutations of the amino acid sequence provided in SEQ ID NO: 5, or a corresponding mutation in any of the amino acid sequences provided in SEQ ID NOs: 6-8. In some embodiments, the GeoCas9 comprises a histidine residue at position 840 of the amino acid sequence provided in SEQ ID NO: 5, or a corresponding mutation in any of the amino acid sequences provided in SEQ ID NOs: 6-8. In some embodiments, the GeoCas9 comprises an amino acid sequence of SEQ ID NO: 7. It is to be understood that other mutations that modify or inactivate the nuclease domains of GeoCas9 may also be included in the dGeoCas9 or GeoCas9n of this disclosure.

[0220] The GeoCas9, GeoCas9n, or dGeoCas9 proteins comprising the mutations disclosed herein, may be a full-length GeoCas9, or a fragment thereof. In some embodiments, proteins comprising GeoCas9, or fragments thereof, are referred to as "GeoCas9 variants." A GeoCas9 variant shares homology to GeoCas9, or a fragment thereof. For example a GeoCas9 variant is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% to a wild-type GeoCas9. In some embodiments, the GeoCas9 variant comprises a fragment of GeoCas9 (e.g., a gRNA binding domain or a DNA-cleavage domain), such that the fragment is at least about 70% identical, at least about 80% identical, at least about 90% identical, at least about 95% identical, at least about 96% identical, at least about 97% identical, at least about 98% identical, at least about 99% identical, at least about 99.5% identical, or at least about 99.9% identical to the corresponding fragment of wild type GeoCas9, e.g., a GeoCas9 comprising the amino acid sequence of SEQ ID NO: 5, SEQ ID NO: 6, or any one of SEQ ID NOs 7-8.

[0221] Any of the GeoCas9 fusion proteins of this disclosure may further comprise an effector domain. In some embodiments, the effector domain is a nucleic acid editing domain (e.g., an enzyme that is capable of modifying nucleic acid, such as a deaminase). In some embodiments, the nucleic acid editing domain is a DNA-editing domain. In some embodiments, the nucleic acid editing domain has deaminase activity. In some embodiments, the nucleic acid editing domain comprises or consists of a deaminase or deaminase domain.

[0222] In some embodiments, the deaminase is a cytidine deaminase. In some embodiments, the deaminase is an apolipoprotein B mRNA-editing complex (APOBEC) family deaminase. In some embodiments, the deaminase is an APOBEC1 family deaminase. In some embodiments, the deaminase is an activation-induced cytidine deaminase (AID). Some nucleic-acid editing domains as well as Cas9

fusion proteins including such domains are described in detail herein. Additional suitable nucleic acid editing domains will be apparent to the skilled artisan based on this disclosure and knowledge in the field.

[0223] Strategies for generating GeoCas9 base editor fusion proteins comprising a GeoCas9 and an effector domain (e.g., a deaminase domain) will be apparent to those of skill in the art based on this disclosure in combination with the general knowledge in the art. Suitable strategies for generating GeoCas9 base editor fusion proteins according to aspects of this disclosure using linkers or without the use of linkers will also be apparent to those of skill in the art in view of the instant disclosure and the knowledge in the art. For example, Gilbert et al., CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell.* 2013; 154 (2): 442-51, showed that C-terminal fusions of Cas9 with VP64 using 2 NLS's as a linker (SPKKKRKVEAS, SEQ ID NO: 23), can be employed for transcriptional activation. Mali et al., CAS9 transcriptional activators for target specificity screening and paired nickases for cooperative genome engineering. Nat Biotechnol. 2013; 31 (9): 833-8, reported that C-terminal fusions with VP64 without linker can be employed for transcriptional activation. And Maeder et al., CRISPR RNA-guided activation of endogenous human genes. *Nat Methods.* 2013; 10:977-979, reported that C-terminal fusions with VP64 using a Gly_4Ser (SEQ ID NO: 10) linker can be used as transcriptional activators. Recently, dCas9-FokI nuclease fusions have successfully been generated and exhibit improved enzymatic specificity as compared to the parental Cas9 enzyme (In Guilinger J P, Thompson D B, Liu D R. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. *Nat. Biotechnol.* 2014; 32 (6): 577-82, and in Tsai S Q, Wyvekens N, Khayter C, Foden J A, Thapar V, Reyon D, Goodwin M J, Aryee M J, Joung J K. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. *Nat Biotechnol.* 2014; 32 (6): 569-76. PMID: 24770325 a SGSETPGTSESATPES (SEQ ID NO: 15) or a $(GGGGS)_n$ (SEQ ID NO: 10), wherein n is 1, linker was used in GeoCas9 base editor fusion proteins, respectively).

[0224] In some embodiments, the various domains of the GeoCas9 fusion proteins can be joined by various linkers that join the domains of the protein, e.g., a first linker that joins a first and second domain, a second linker that joins a second domain to a third domain, etc.

[0225] For example, the first linker can comprise an amino acid sequence of 1-50 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 1-40 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 1-35 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 1-30 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 1-20 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 10-20 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 30-40 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 14, 16, or 18 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 16 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 30, 32, or 34 amino acids. In some embodiments, the first linker comprises an amino acid sequence of 32 amino acids. In some embodiments, the first linker comprises a SGSETPGTSESATPES (SEQ ID NO: 15) motif. In some embodiments, the first linker comprises a SGGSSGGSSG-SETPGTSESATPESSGGSSGGS (SEQ ID NO: 17) motif.

[0226] In some embodiments, the second linker comprises comprises an amino acid sequence of 1-50 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 1-40 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 1-35 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 1-30 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 1-20 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 2-20 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 2-10 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 10-20 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 2, 4, or 6 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 7, 9, or 11 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 14, 16, or 18 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 4 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 9 amino acids. In some embodiments, the second linker comprises an amino acid sequence of 16 amino acids. In some embodiments, the second linker comprises a $(SGGS)_n$ (SEQ ID NO: 14) motif, wherein n is an integer between 1 and 30, inclusive. In some embodiments, the second linker comprises a $(SGGS)_n$ (SEQ ID NO: 14) motif, wherein n is 1. In some embodiments, the second linker comprises a SGGS $(GGS)_n$ (SEQ ID NO: 16) motif, wherein n is an integer between 1 and 30, inclusive. In some embodiments, the second linker comprises a SGGS$(GGS)_n$ (SEQ ID NO: 16) motif, wherein n is 2.

[0227] In some embodiments, the third linker comprises an amino acid sequence of 1-50 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 1-40 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 1-35 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 1-30 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 1-20 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 2-20 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 2-10 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 10-20 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 2, 4, or 6 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 7, 9, or 11 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 14, 16, or 18 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 4 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 9 amino acids. In some embodiments, the third linker comprises an amino acid sequence of 16 amino acids. In some embodiments, the third linker comprises a $(SGGS)_n$ (SEQ ID NO: 14) motif, wherein n is an integer between 1 and 30, inclusive. In some embodiments, the third linker comprises a $(SGGS)_n$ (SEQ

ID NO: 14) motif, wherein n is 1. In some embodiments, the third linker comprises a SGGS(GGS)$_n$ (SEQ ID NO: 16) motif, wherein n is an integer between 1 and 30, inclusive. In some embodiments, the third linker comprises a SGGS (GGS)$_n$ (SEQ ID NO: 16) motif, wherein n is 2.

[0228] In some embodiments, the GeoCas9 base editor fusion proteins provided herein do not comprise a linker sequence. In some embodiments, one or both of the optional linker sequences are present. In some embodiments, one, two, or three of the optional linker sequences are present.

[0229] In some embodiments, the "-" used in the general architecture above indicates the presence of an optional linker sequence. In some embodiments, the GeoCas9 base editor fusion proteins comprising a UGI further comprise a nuclear targeting sequence, for example a nuclear localization sequence. In some embodiments, GeoCas9 base editor fusion proteins provided herein further comprise a nuclear localization sequence (NLS). In some embodiments, the NLS is fused to the N-terminus of the GeoCas9 base editor fusion protein. In some embodiments, the NLS is fused to the C-terminus of the GeoCas9 base editor fusion protein. In some embodiments, the NLS is fused to the N-terminus of the UGI protein. In some embodiments, the NLS is fused to the C-terminus of the UGI protein. In some embodiments, the NLS is fused to the N-terminus of the Cas9 protein. In some embodiments, the NLS is fused to the C-terminus of the Cas9 protein. In some embodiments, the NLS is fused to the N-terminus of the deaminase. In some embodiments, the NLS is fused to the C-terminus of the deaminase. In some embodiments, the NLS is fused to the N-terminus of the second Cas9. In some embodiments, the NLS is fused to the C-terminus of the second Cas9. In some embodiments, the NLS is fused to the GeoCas9 base editor fusion protein via one or more linkers. In some embodiments, the NLS is fused to the fusioin protein without a linker. In some embodiments, the NLS comprises an amino acid sequence of any one of the NLS sequences provided or referenced herein. In some embodiments, the NLS comprises an amino acid sequence as set forth in SEQ ID NO: 75 or SEQ ID NO: 76.

[0230] In a preferred embodiment, the GeoCas9 base editor fusion protein may comprise the following structures:

[0231] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[UGI domain]-[COOH];

[0232] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[UGI domain]-[NLS]-[COOH];

[0233] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[COOH]; or

[0234] [NH^2]-[nucleic acid effector domain]-[GeoCas9]-[first UGI domain]-[second UGI domain]-[NLS]-[COOH].

[0235] In some embodiments, the nucleic acid effector domain and the GeoCas9 are fused via a linker comprising or consisting of the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 16), also referred to as the "XTEN linker". In some embodiments, the nucleic acid effector domain and the GeoCas9 are fused via a linker comprising or consisting of the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 17).

[0236] In some embodiments, the GeoCas9 and the UGI domain are vused via a linker comprising or consisting of the amino acid sequence SGGS (SEQ ID NO: 14). In some embodiments, the GeoCas9 and the first UGI domain are vused via a linker comprising or consisting of the amino acid sequence SGGS (SEQ ID NO: 14). In some embodiments, the first UGI domain and the second UGI domain are fused via a linker comprising or consisting of the amino acid sequence SGGSGGSGGS (SEQ ID NO: 24). In some embodiments, the cytidine deaminase domain and the GeoCas9 are fused via a linker comprising or consisting of the amino acid sequence SGSETPGTSESATPES (SEQ ID NO: 15), and the GeoCas9 and the UGI domain are fused via a linker comprising or consisting of the amino acid sequence SGGS (SEQ ID NO: 14). In some embodiments, the nucleic acid effector domain and the GeoCas9 are fused via a linker comprising or consisting of the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 17), the GeoCas9 and the first UGI domain are fused via a linker comprising or consisting of the amino acid sequence SGGS (SEQ ID NO: 14), and the first UGI domain and the second UGI domain are fused via a linker comprising or consisting of the amino acid sequence SGGSGGSGGS (SEQ ID NO: 24)

[0237] In the GeoCas9 base editor fusion protein structures provided above, NLS is a nuclear localization sequence, NH_2 is the N-terminus of the GeoCas9 base editor fusion protein, COOH is the C-terminus of the GeoCas9 base editor fusion protein, and each instance of "-" comprises an optional linker, for example any of the linkers provided herein.

[0238] It should be appreciated that any of the GeoCas9 base editor fusion proteins described above may be comprised of (i) a GeoCas9 (e.g., a nGeoCas9 or dGeoCas9); (ii) an effector domain (e.g., cytidine or adenosine deaminase); and optionally (iii) two or more UGI domains, wherein the two or more UGI domains may be adjacent (e.g., [first UGI domain]-[second UGI domain], wherein "-" is an optional linker) to one another in the construct, or the two or more UGI domains may be separated by the GeoCas9 of (i) and/or the cytidine deaminase domain of (ii) (e.g., [first UGI domain]-[deaminase domain]-[second UGI domain], [first UGI domain]-[napDNAbp]-[second UGI domain], [first UGI domain]-[deaminase domain]-[napDNAbp]-[second UGI domain], ect., wherein "-" is an optional linker).

[0239] In another aspect, the GeoCas9 base editor fusion protein comprises: (i) a GeoCas9; (ii) a nucleic acid-editing enzyme or domain (e.g., a second protein, such as a cytidine deaminase domain); (iii) a first uracil glycosylase inhibitor domain (UGI) (e.g., a third protein); and (iv) a second uracil glycosylase inhibitor domain (UGI) (e.g., a fourth protein). The first and second uracil glycosylase inhibitor domains (UGIs) may be the same or different. In some embodiments, the GeoCas9 (e.g., the first protein) and the deaminase (e.g., the second protein) are fused via a linker. In some embodiments, the GeoCas9 is fused to the C-terminus of the deaminase. In some embodiments, the GeoCas9 protein (e.g., the first protein) and the first UGI domain (e.g., the third protein) are fused via a linker (e.g., a second linker). In some embodiments, the first UGI domain is fused to the C-terminus of the GeoCas9 protein. In some embodiments, the first UGI domain (e.g., the third protein) and the second UGI domain (e.g., the forth protein) are fused via a linker (e.g., a third linker). In some embodiments, the second UGI domain is fused to the C-terminus of the first UGI domain. In some embodiments, the linker comprises a (GGGS)$_n$ (SEQ ID NO: 9), (GGGGS)$_n$ (SEQ ID NO: 10), a (G)$_n$ (SEQ ID NO: 11), an (EAAAK)$_n$ (SEQ ID NO: 12), a (GGS)$_n$ (SEQ ID NO: 13), (SGGS)$_n$ (SEQ ID NO: 14), a

SGSETPGTSESATPES (SEQ ID NO: 15), a SGGS $(GGS)_n$ (SEQ ID NO: 16), a SGGSSGGSSGSETPGTSESATPESSGGSSGGS (SEQ ID NO: 17), or an $(XP)_n$ (SEQ ID NO: 18) motif, or a combination of any of these, wherein n is independently an integer between 1 and 30.

II. Guide RNA

[0240] In various embodiments, the GeoCas9 base editors described herein may be complexed, bound, or otherwise associated with (e.g., via any type of covalent or non-covalent bond) one or more guide sequences, i.e., the sequence which becomes associated or bound to the base editor and directs its localization to a specific target sequence having complementarity to the guide sequence or a portion thereof. The particular design embodiments of a guide sequence will depend upon the nucleotide sequence of a genomic target site of interest (i.e., the desired site to be edited) and the particular GeoCas9 protein present in the base editor, among other factors, such as PAM sequence locations, percent G/C content in the target sequence, the degree of microhomology regions, secondary structures, etc.

[0241] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a GeoCas9 to a target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length.

[0242] In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of a base editor to a target sequence may be assessed by any suitable assay. For example, the components of a base editor, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of a base editor disclosed herein, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a base editor, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.

[0243] In some embodiments, a guide sequence is selected to reduce the degree of secondary structure within the guide sequence. Secondary structure may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is mFold, as described by Zuker & Stiegler (*Nucleic Acids Res.* 9 (1981), 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see, e.g., A. R. Gruber et al., 2008, *Cell* 106 (1): 23-24; and P A Carr & GM Church, 2009, *Nature Biotechnology* 27 (12): 1151-62). Additional algorithms may be found in Chuai, G. et al., *DeepCRISPR: optimized CRISPR guide RNA design by deep learning, Genome Biol.* 19:80 (2018), and U.S. application Ser. No. 61/836,080, the entireties of each of which are incorporated herein by reference.

[0244] The guide sequence can be linked to a tracr mate sequence which in turn hybridizes to a tracr sequence. A tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the corresponding tracr sequence; and (2) formation of a complex at a target sequence, wherein the complex comprises the tracr mate sequence hybridized to the tracr sequence. In general, degree of complementarity is with reference to the optimal alignment of the tracr mate sequence and tracr sequence, along the length of the shorter of the two sequences. Optimal alignment may be determined by any suitable alignment algorithm, and may further account for secondary structures, such as self-complementarity within either the tracr sequence or tracr mate sequence.

[0245] In some embodiments, the degree of complementarity between the tracr sequence and tracr mate sequence along the length of the shorter of the two when optimally aligned is about or more than about 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99%, or higher. In some embodiments, the tracr sequence is about or more than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or more nucleotides in length. In some embodiments, the tracr sequence and tracr mate sequence are contained within a single transcript, such that hybridization between the two produces a transcript having a secondary structure, such as a hairpin. Preferred loop forming sequences for use in hairpin structures are four nucleotides in length, and most preferably have the sequence GAAA. However, longer or shorter loop sequences may be used, as may alternative sequences. The sequences preferably include a nucleotide triplet (for example, AAA), and an additional nucleotide (for example C or G). Examples of loop forming sequences include CAAA and AAAG. In an embodiment of the disclosure, the transcript or transcribed polynucleotide sequence has at least two or more hairpins. In certain embodiments, the transcript has two, three, four or five hairpins. In a further embodiment of the disclosure, the transcript has at most five hairpins. In some embodiments, the single transcript further includes a transcription termination sequence; preferably this is a polyT sequence, for example six T nucleotides. Further non-limiting examples of

single polynucleotides comprising a guide sequence, a tracr mate sequence, and a tracr sequence are as follows (listed 5' to 3'), where "N" represents a base of a guide sequence, the first block of lower case letters represent the tracr mate sequence, and the second block of lower case letters represent the tracr sequence, and the final poly-T sequence represents the transcription terminator:

(SEQ ID NO: 376)
(1) NNNNNNNNgtttttgtactctcaagatttaGAAAtaaatcttgcag aagctacaaagataaggcttcatgccgaaatcaacaccctgtcattttat ggcagggtgttttcgttatttaaTTTTTT;

(SEQ ID NO: 377)
(2)NNNNNNNNNNNNNNNNNNgtttttgtactctcaGAAAtgcagaagct acaaagataaggcttcatgccgaaatca acaccctgtcattttatggca gggtgttttcgttatttaaTTTTTT;

(SEQ ID NO: 378)
(3)NNNNNNNNNNNNNNNNNNNNgtttttgtactctcaGAAAtgcagaag ctacaaagataaggcttcatgccgaaatca acaccctgtcattttatgg cagggtgtTTTTT;

(SEQ ID NO: 379)
(4)NNNNNNNNNNNNNNNNNNNNgttttagagctaGAAAtagcaagttaa aataaggctagtccgttatcaacttgaaaa agtggcaccgagtcggtgc TTTTTT;

(SEQ ID NO: 380)
(5)NNNNNNNNNNNNNNNNNNNNgttttagagctaGAAATAGcaagttaa aataaggctagtccgttatcaacttgaa aaagtgTTTTTTT;
and

(SEQ ID NO: 381)
(6)NNNNNNNNNNNNNNNNNNNNgttttagagctagAAATAGcaagttaa aataaggctagtccgttatcaTTTTT TTT.

[0246] In some embodiments, the tracr sequence is a separate transcript from a transcript comprising the tracr mate sequence.

[0247] It will be apparent to those of skill in the art that in order to target any of the herein disclosed GeoCas9 fusion proteins to a target site, e.g., a site comprising a point mutation to be edited, it is typically necessary to co-express the fusion protein together with a guide RNA. A guide RNA typically comprises a tracrRNA framework allowing for GeoCas9 binding, and a guide sequence or spacer sequence, which confers sequence specificity to the Cas9: nucleic acid editing enzyme/domain fusion protein.

[0248] In some embodiments, the guide RNA comprises a structure 5'-[guide sequence]-guuuuagagcuagaaauagcaaguuaaaauaaggcuaguccguuaucaacuugaaaaaguggcaccgagucggugcuuu uu-3' (SEQ ID NO: 382), wherein the guide sequence comprises a sequence that is complementary to the target sequence. See U.S. Publication No. 2015/0166981, published Jun. 18, 2015, the disclosure of which is incorporated by reference herein in its entirety. The guide sequence is typically 20 nucleotides long.

[0249] The sequences of suitable guide RNAs for targeting GeoCas9: nucleic acid editing enzyme/domain fusion proteins to specific genomic target sites will be apparent to those of skill in the art based on the instant disclosure. Such suitable guide RNA sequences typically comprise guide sequences that are complementary to a nucleic sequence within 50 nucleotides upstream or downstream of the target nucleotide to be edited. Some exemplary guide RNA sequences suitable for targeting any of the provided fusion proteins to specific target sequences are provided herein.

[0250] Additional guide sequences are well known in the art and can be used with the base editors described herein. Additional exemplary guide sequences are disclosed in, for example, Jinek M., et al., *Science* 337:816-821 (2012); Mali P, Esvelt K M & Church G M (2013) Cas9 as a versatile tool for engineering biology, Nature Methods, 10, 957-963; Li J F et al., (2013) Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9, *Nature Biotechnology,* 31, 688-691; Hwang, W. Y. et al., Efficient genome editing in zebrafish using a CRISPR-Cas system, *Nature Biotechnology* 31, 227-229 (2013); Cong L et al., (2013) Multiplex genome engineering using CRIPSR/Cas systems, *Science,* 339, 819-823; Cho S W et al., (2013) Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease, *Nature Biotechnology,* 31, 230-232; Jinek, M. et al., RNA-programmed genome editing in human cells, *eLife* 2, e00471 (2013); Dicarlo, J. E. et al., Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. Nucleic Acid Res. (2013); Briner A E et al., (2014) Guide RNA functional modules direct Cas9 activity and orthogonality, *Mol Cell,* 56, 333-339, the entire contents of each of which are herein incorporated by reference.

III. GeoCas9 BE/Guide RNA Complexes

[0251] Some aspects of this disclosure provide complexes comprising any of the GeoCas9 base editor fusion proteins provided herein and a guide RNA.

[0252] In various embodiments, the guide RNA is bound to a GeoCas9 protein (e.g., a dGeoCas9, a nuclease active GeoCas9, or a GeoCas9 nickase) of GeoCas9 base editor fusion protein and functions to guide the GeoCas9 fusion protein to a target nucleotide sequence.

[0253] In some embodiments, the guide RNA is from 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence. In some embodiments, the guide RNA is 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides long. In some embodiments, the guide RNA comprises a sequence of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 contiguous nucleotides that is complementary (e.g., perfectly complementary) to a target sequence. In some embodiments, the target sequence is a DNA sequence. In some embodiments, the target sequence is a sequence in the genome of a mammal. In some embodiments, the target sequence is a sequence in the genome of a human. In some embodiments, the 3' end of the target sequence is immediately adjacent to a PAM sequence (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)), such as any of the PAM sequences provided herein. In some embodiments, the guide RNA is complementary to a sequence associated with a disease or disorder.

[0254] In some embodiments, the target sequence comprises a sequence associated with a disease or disorder. In some embodiments, the target sequence comprises a point mutation associated with a disease or disorder. In some embodiments, the target sequence comprises a T→C point mutation. In some embodiments, the complex deaminates the target C point mutation, wherein the deamination results in a sequence that is not associated with a disease or disorder. In some embodiments, the target C point mutation is present in the DNA strand that is not complementary to the guide RNA. In some embodiments, the target sequence comprises a T->A point mutation. In some embodiments, the complex deaminates the target A point mutation, and wherein the deamination results in a sequence that is not associated with a disease or disorder. In some embodiments, the target A point mutation is present in the DNA strand that is not complementary to the guide RNA.

[0255] In some embodiments, the complex edits a point mutation in the target sequence. In some embodiments, the point mutation is located between about 10 to about 20 nucleotides upstream of the PAM in the target sequence. In some embodiments, the point mutation is located between about 13 to about 17 nucleotides upstream of the PAM in the target sequence. In some embodiments, the point mutation is about 13 nucleotides upstream of the PAM. In some embodiments, the point mutation is about 14 nucleotides upstream of the PAM. In some embodiments, the point mutation is about 15 nucleotides upstream of the PAM. In some embodiments, the point mutation is about 16 nucleotides upstream of the PAM. In some embodiments, the point mutation is about 17 nucleotides upstream of the PAM.

[0256] In some embodiments, the complex exhibits increased deamination efficiency of a point mutation in a target sequence that does not comprise the canonical PAM (5'-NGG-3') at its 3' end as compared to the deamination efficiency of a complex comprising *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1. In some embodiments, the complex exhibits increased deamination efficiency of a point mutation in a target sequence having a 3' end that is not directly adjacent to the canonical PAM sequence (5'-NGG-3') that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold increased as compared to the deamination efficiency of complex comprising the *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1 on the same target sequence. In some embodiments, the 3' end of the target sequence is directly adjacent to a sequence selected from the group consisting of NGT, NGA, NGC, and NNG, wherein N is an A, G, T, or C. In some embodiments, the 3' end of the target sequence is directly adjacent to a sequence selected from the group consisting of CGG, AGT, TGG, AGT, CGT, GGG, CGT, TGT, GGT, AGC, CGC, TGC, GGC, AGA, CGA, TGA, GGA, GAA, GAT, and CAA. In some embodiments, deamination activity is measured using high-throughput sequencing.

[0257] In some embodiments, the complex produces fewer indels in a target sequence that does not comprise the canonical PAM (5'-NGG-3') at its 3' end as compared to the amount of indels produced by a complex comprising *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1. In some embodiments, the complex produces fewer indels in a target sequence having a 3' end that is not directly adjacent to the canonical PAM sequence (5'-NGG-3') that is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold lower as compared to the amount of indels produced by a complex comprising *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1 on the same target sequence. In some embodiments, the 3' end of the target sequence is directly adjacent to a sequence selected from the group consisting of NGT, NGA, NGC, and NNG, wherein N is an A, G, T, or C. In some embodiments, the 3' end of the target sequence is directly adjacent to a sequence selected from the group consisting of CGG, AGT, TGG, AGT, CGT, GGG, CGT, TGT, GGT, AGC, CGC, TGC, GGC, AGA, CGA, TGA, GGA, GAA, GAT, and CAA. In some embodiments, indels are measured using high-throughput sequencing.

[0258] In some embodiments, the complex exhibits a decreased off-target activity as compared to the off-target activity of a complex comprising *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1. In some embodiments, the off-target activity of the complex is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1,000-fold, at least 5,000-fold, at least 10,000-fold, at least 50,000-fold, at least 100,000-fold, at least 500,000-fold, or at least 1,000,000-fold decreased as compared to the off-target activity of a complex comprising *Streptococcus pyogenes* Cas9 as provided by SEQ ID NO: 1. In some embodiments, the off-target activity is determined using a genome-wide off-target analysis. In some embodiments, the off-target activity is determined using GUIDE-seq.

IV. Methods of Using GeoCas9 Fusion Proteins

[0259] Some aspects of this disclosure provide methods of using the GeoCas9 proteins, fusion proteins, or complexes provided herein. For example, some aspects of this disclosure provide methods comprising contacting a DNA molecule (a) with any of the the GeoCas9 proteins or fusion proteins provided herein, and with at least one guide RNA, wherein the guide RNA is about 15-100 nucleotides long and comprises a sequence of at least 10 contiguous nucleotides that is complementary to a target sequence; or (b) with a GeoCas9 protein, a GeoCas9 fusion protein, or a GeoCas9 protein or fusion protein complex with at least one gRNA as provided herein. In some embodiments, the 3' end of the target sequence is not immediately adjacent to a canonical PAM sequence (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)), sush as any of the PAM sequences provided herein.

[0260] In some embodiments, the target DNA sequence comprises a sequence associated with a disease or disorder. In some embodiments, the target DNA sequence comprises a point mutation associated with a disease or disorder. In some embodiments, the activity of the Cas9 protein, the Cas9 fusion protein, or the complex results in a correction of the point mutation. In some embodiments, the target DNA sequence comprises a T->C point mutation associated with a disease or disorder, and wherein the deamination of the mutant C base results in a sequence that is not associated with a disease or disorder. In some embodiments, the target DNA sequence encodes a protein and wherein the point mutation is in a codon and results in a change in the amino

acid encoded by the mutant codon as compared to the wild-type codon. In some embodiments, the deamination of the mutant C results in a change of the amino acid encoded by the mutant codon. In some embodiments, the deamination of the mutant C results in the codon encoding the wild-type amino acid. In some embodiments, the contacting is in vivo in a subject. In some embodiments, the subject has or has been diagnosed with a disease or disorder. In some embodiments, the disease or disorder is cystic fibrosis, phenylketonuria, epidermolytic hyperkeratosis (EHK), Charcot-Marie-Toot disease type 4J, neuroblastoma (NB), von Willebrand disease (vWD), myotonia congenital, hereditary renal amyloidosis, dilated cardiomyopathy (DCM), hereditary lymphedema, familial Alzheimer's disease, HIV, Prion disease, chronic infantile neurologic cutaneous articular syndrome (CINCA), desmin-related myopathy (DRM), a neoplastic disease associated with a mutant PI3KCA protein, a mutant CTNNB 1 protein, a mutant HRAS protein, or a mutant p53 protein.

[0261] In some embodiments, any of the base editors provided herein may be used to treat a disease or disorder. For example, any base editors provided herein may be used to correct one or more mutations associated with any of the diseases or disorders provided herein. Exemplary diseases or disorders that may be treated include, without limitation, those listed in Table 1 and Table 2, as well as 3-Methylglutaconic aciduria type 2, 46,XY gonadal dysgenesis, 4-Alpha-hydroxyphenylpyruvate hydroxylase deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, achromatopsia, Acid-labile subunit deficiency, Acrodysostosis, acroerythrokeratoderma, ACTH resistance, ACTH-independent macronodular adrenal hyperplasia, Activated PI3K-delta syndrome, Acute intermittent porphyria, Acute myeloid leukemia, Adams-Oliver syndrome 1/5/6, Adenylosuccinate lyase deficiency, Adrenoleukodystrophy, Adult neuronal ceroid lipofuscinosis, Adult onset ataxia with oculomotor apraxia, Advanced sleep phase syndrome, Age-related macular degeneration, Alagille syndrome, Alexander disease, Allan-Herndon-Dudley syndrome, Alport syndrome, X-linked recessive, Alternating hemiplegia of childhood, Alveolar capillary dysplasia with misalignment of pulmonary veins, Amelogenesis imperfecta, Amyloidogenic transthyretin amyloidosis, Amyotrophic lateral sclerosis, Anemia (nonspherocytic hemolytic, due to G6PD deficiency), Anemia (sideroblastic, pyridoxine-refractory, autosomal recessive), Anonychia, Antithrombin III deficiency, Aortic aneurysm, Aplastic anemia, Apolipoprotein C2 deficiency, Apparent mineralocorticoid excess, Aromatase deficiency, Arrhythmogenic right ventricular cardiomyopathy, Familial hypertrophic cardiomyopathy, Hypertrophic cardiomyopathy, Arthrogryposis multiplex congenital, Aspartylglycosaminuria, Asphyxiating thoracic dystrophy, Ataxia with vitamin E deficiency, Ataxia (spastic), Atrial fibrillation, Atrial septal defect, atypical hemolytic-uremic syndrome, autosomal dominant CD11C+/CD1C+ dendritic cell deficiency, Autosomal dominant progressive external ophthalmoplegia with mitochondrial DNA deletions, Baraitser-Winter syndrome, Bartter syndrome, Basa ganglia calcification, Beckwith-Wiedemann syndrome, Benign familial neonatal seizures, Benign scapuloperoneal muscular dystrophy, Bernard Soulier syndrome, Beta thalassemia intermedia, Beta-D-mannosidosis, Bietti crystalline corneoretinal dystrophy, Bile acid malabsorption, Biotinidase deficiency, Borjeson-Forssman-Lehmann syndrome, Boucher Neuhauser syndrome, Bowen-Conradi syndrome, Brachydactyly, Brown-Vialetto-Van laere syndrome, Brugada syndrome, Cardiac arrhythmia, Cardiofaciocutaneous syndrome, Cardiomyopathy, Carnevale syndrome, Carnitine palmitoyltransferase II deficiency, Carpenter syndrome, Cataract, Catecholaminergic polymorphic ventricular tachycardia, Central core disease, Centromeric instability of chromosomes 1,9 and 16 and immunodeficiency, Cerebral autosomal dominant arteriopathy, Cerebro-oculo-facio-skeletal syndrome, Ceroid lipofuscinosis, Charcot-Marie-Tooth disease, Cholestanol storage disease, Chondrocalcinosis, Chondrodysplasia, Chronic progressive multiple sclerosis, Coenzyme Q10 deficiency, Cohen syndrome, Combined deficiency of factor V and factor VIII, Combined immunodeficiency, Combined oxidative phosphorylation deficiency, Combined partial 17-alpha-hydroxylase/17,20-lyase deficiency, Complement factor d deficiency, Complete combined 17-alpha-hydroxylase/17,20-lyase deficiency, Conerod dystrophy, Congenital contractural arachnodactyly, Congenital disorder of glycosylation, Congenital lipomatous overgrowth, Neoplasm of ovary, PIK3CA Related Overgrowth Spectrum, Congenital long QT syndrome, Congenital muscular dystrophy, Congenital muscular hypertrophy-cerebral syndrome, Congenital myasthenic syndrome, Congenital myopathy with fiber type disproportion, Eichsfeld type congenital muscular dystrophy, Congenital stationary night blindness, Corneal dystrophy, Cornelia de Lange syndrome, Craniometaphyseal dysplasia, Crigler Najjar syndrome, Crouzon syndrome, Cutis laxa with osteodystrophy, Cyanosis, Cystic fibrosis, Cystinosis, Cytochrome-c oxidase deficiency, Mitochondrial complex I deficiency, D-2-hydroxyglutaric aciduria, Danon disease, Deafness with labyrinthine aplasia microtia and microdontia (LAMM), Deafness, Deficiency of acetyl-CoA acetyltransferase, Deficiency of ferroxidase, Deficiency of UDPglucose-hexose-1-phosphate uridylyltransferase, Dejerine-Sottas disease, Desbuquois syndrome, DFNA, Diabetes mellitus type 2, Diabetes-deafness syndrome, Diamond-Blackfan anemia, Diastrophic dysplasia, Dihydropteridine reductase deficiency, Dihydropyrimidinase deficiency, Dilated cardiomyopathy, Disseminated atypical mycobacterial infection, Distal arthrogryposis, Distal hereditary motor neuronopathy, Donnai Barrow syndrome, Duchenne muscular dystrophy, Becker muscular dystrophy, Dyschromatosis universalis hereditaria, Dyskeratosis congenital, Dystonia, Early infantile epileptic encephalopathy, Ehlers-Danlos syndrome, Eichsfeld type congenital muscular dystrophy, Emery-Dreifuss muscular dystrophy, Enamel-renal syndrome, Epidermolysis bullosa dystrophica inversa, Epidermolysis bullosa herpetiformis, Epilepsy, Episodic ataxia, Erythrokeratodermia variabilis, Erythropoietic protoporphyria, Exercise intolerance, Exudative vitreoretinopathy, Fabry disease, Factor V deficiency, Factor VII deficiency, Factor xiii deficiency, Familial adenomatous polyposis, breast cancer, ovarian cancer, cold urticarial, chronic infantile neurological, cutaneous and articular syndrome, hemiplegic migraine, hypercholesterolemia, hypertrophic cardiomyopathy, hypoalphalipoproteinemia, hypokalemia-hypomagnesemia, juvenile gout, hyperlipoproteinemia, visceral amyloidosis, hypophosphatemic vitamin D refractory rickets, FG syndrome, Fibrosis of extraocular muscles, Finnish congenital nephrotic syndrome, focal epilepsy, Focal segmental glomerulosclerosis, Frontonasal dysplasia, Frontotemporal dementia, Fructose-biphosphatase deficiency, Gamstorp-Wohlfart syn-

drome, Ganglioside sialidase deficiency, GATA-1-related thrombocytopenia, Gaucher disease, Giant axonal neuropathy, Glanzmann thrombasthenia, Glomerulocystic kidney disease, Glomerulopathy, Glucocorticoid resistance, Glucose-6-phosphate transport defect, Glutaric aciduria, Glycogen storage disease, Gorlin syndrome, Holoprosencephaly, GRACILE syndrome, Hemorrhagic telangiectasia, Hemochromatosis, Hemoglobin H disease, Hemolytic anemia, Hemophagocytic lymphohistiocytosis, Carcinoma of colon, Myhre syndrome, leukoencephalopathy, Hereditary factor IX deficiency disease, Hereditary factor VIII deficiency disease, Hereditary factor XI deficiency disease, Hereditary fructosuria, Hereditary Nonpolyposis Colorectal Neoplasm, Hereditary pancreatitis, Hereditary pyropoikilocytosis, Elliptocytosis, Heterotaxy, Heterotopia, Histiocytic medullary reticulosis, Histiocytosis-lymphadenopathy plus syndrome, HNSHA due to aldolase A deficiency, Holocarboxylase synthetase deficiency, Homocysteinemia, Howel-Evans syndrome, Hydatidiform mole, Hypercalciuric hypercalcemia, Hyperimmunoglobulin D, Mevalonic aciduria, Hyperinsulinemic hypoglycemia, Hyperkalemic Periodic Paralysis, Paramyotonia congenita of von Eulenburg, Hyperlipoproteinemia, Hypermanganesemia, Hypermethioninemia, Hyperphosphatasemia, Hypertension, hypomagnesemia, Hypobetalipoproteinemia, Hypocalcemia, Hypogonadotropic hypogonadism, Hypogonadotropic hypogonadism, Hypohidrotic ectodermal dysplasia, Hyper-IgM immunodeficiency, Hypohidrotic X-linked ectodermal dysplasia, Hypomagnesemia, Hypoparathyroidism, Idiopathic fibrosing alveolitis, Immunodeficiency, Immunoglobulin A deficiency, Infantile hypophosphatasia, Infantile Parkinsonism-dystonia, Insulin-dependent diabetes mellitus, Intermediate maple syrup urine disease, Ischiopatellar dysplasia, Islet cell hyperplasia, Isolated growth hormone deficiency, Isolated lutropin deficiency, Isovaleric acidemia, Joubert syndrome, Juvenile polyposis syndrome, Juvenile retinoschisis, Kallmann syndrome, Kartagener syndrome, Kugelberg-Welander disease, Lattice corneal dystrophy, Leber congenital amaurosis, Leber optic atrophy, Left ventricular noncompaction, Leigh disease, Mitochondrial complex I deficiency, Leprechaunism syndrome, Arthrogryposis, Anterior horn cell disease, Leukocyte adhesion deficiency, Leukodystrophy, Leukoencephalopathy, Ovarioleukodystrophy, L-ferritin deficiency, Li-Fraumeni syndrome, Limb-girdle muscular dystrophy-dystroglycanopathy, Loeys-Dietz syndrome, Long QT syndrome, Macrocephaly/autism syndrome, Macular corneal dystrophy, Macular dystrophy, Malignant hyperthermia susceptibility, Malignant tumor of prostate, Maple syrup urine disease, Marden Walker like syndrome, Marfan syndrome, Marie Unna hereditary hypotrichosis, Mast cell disease, Meconium ileus, Medium-chain acyl-coenzyme A dehydrogenase deficiency, Melnick-Fraser syndrome, Mental retardation, Merosin deficient congenital muscular dystrophy, Mesothelioma, Metachromatic leukodystrophy, Metaphyseal chondrodysplasia, Methemoglobinemia, methylmalonic aciduria, homocystinuria, Microcephaly, chorioretinopathy, lymphedema, Microphthalmia, Mild non-PKU hyperphenylalanemia, Mitchell-Riley syndrome, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase deficiency, Mitochondrial complex I deficiency, Mitochondrial complex III deficiency, Mitochondrial myopathy, Mucolipidosis III, Mucopolysaccharidosis, Multiple sulfatase deficiency, Myasthenic syndrome, *Mycobacterium tuberculosis*, Myeloperoxidase deficiency, Myhre syndrome, Myoclonic epilepsy, Myofibrillar myopathy, Myoglobinuria, Myopathy, Myopia, Myotonia congenital, Navajo neurohepatopathy, Nemaline myopathy, Neoplasm of stomach, Nephrogenic diabetes insipidus, Nephronophthisis, Nephrotic syndrome, Neurofibromatosis, Neutral lipid storage disease, Niemann-Pick disease, Non-ketotic hyperglycinemia, Noonan syndrome, Noonan syndrome-like disorder, Norum disease, Macular degeneration, N-terminal acetyltransferase deficiency, Oculocutaneous albinism, Oculodentodigital dysplasia, Ohdo syndrome, Optic nerve aplasia, Ornithine carbamoyltransferase deficiency, Orofaciodigital syndrome, Osteogenesis imperfecta, Osteopetrosis, Ovarian dysgenesis, Pachyonychia, Palmoplantar keratoderma, nonepidermolytic, Papillon-Lef\xc3\xa8vre syndrome, Haim-Munk syndrome, Periodontitis, Peeling skin syndrome, Pendred syndrome, Peroxisomal fatty acyl-coa reductase 1 disorder, Peroxisome biogenesis disorder, Pfeiffer syndrome, Phenylketonuria, Phenylketonuria, Hyperphenylalaninemia, non-PKU, Pituitary hormone deficiency, *Pityriasis rubra* pilaris, Polyarteritis nodosa, Polycystic kidney disease, Polycystic lipomembranous osteodysplasia, Polymicrogyria, Pontocerebellar hypoplasia, Porokeratosis, Posterior column ataxia, Primary erythromelalgia, hyperoxaluria, Progressive familial intrahepatic cholestasis, Progressive pseudorheumatoid dysplasia, Propionic acidemia, Pseudohermaphroditism, Pseudohypoaldosteronism, Pseudoxanthoma elasticum-like disorder, Purine-nucleoside phosphorylase deficiency, Pyridoxal 5-phosphate-dependent epilepsy, Renal dysplasia, retinal pigmentary dystrophy, cerebellar ataxia, skeletal dysplasia, Reticular dysgenesis, Retinitis pigmentosa, Usher syndrome, Retinoblastoma, Retinopathy, RRM2B-related mitochondrial disease, Rubinstein-Taybi syndrome, Schnyder crystalline corneal dystrophy, Sebaceous tumor, Severe congenital neutropenia, Severe myoclonic epilepsy in infancy, Severe X-linked myotubular myopathy, onychodysplasia, facial dysmorphism, hypotrichosis, Short-rib thoracic dysplasia, Sialic acid storage disease, Sialidosis, Sideroblastic anemia, Small fiber neuropathy, Smith-Magenis syndrome, Sorsby fundus dystrophy, Spastic ataxia, Spastic paraplegia, Spermatogenic failure, Spherocytosis, Sphingomyelin/cholesterol lipidosis, Spinocerebellar ataxia, Split-hand/foot malformation, Spondyloepimetaphyseal dysplasia, Platyspondylic lethal skeletal dysplasia, Squamous cell carcinoma of the head and neck, Stargardt disease, Sucrase-isomaltase deficiency, Sudden infant death syndrome, Supravalvar aortic stenosis, Surfactant metabolism dysfunction, Tangier disease, Tatton-Brown-rahman syndrome, Thoracic aortic aneurysms and aortic dissections, Thrombophilia, Thyroid hormone resistance, TNF receptor-associated periodic fever syndrome (TRAPS), Tooth agenesis, Torsades de pointes, Transposition of great arteries, Treacher Collins syndrome, Tuberous sclerosis syndrome, Tyrosinase-negative oculocutaneous albinism, Tyrosinase-positive oculocutaneous albinism, Tyrosinemia, UDPglucose-4-epimerase deficiency, Ullrich congenital muscular dystrophy, Bethlem myopathy Usher syndrome, UV-sensitive syndrome, Van der Woude syndrome, popliteal pterygium syndrome, Very long chain acyl-CoA dehydrogenase deficiency, Vesicoureteral reflux, Vitreoretinochoroidopathy, Von Hippel-Lindau syndrome, von Willebrand disease, Waardenburg syndrome, Warsaw breakage syndrome, WFS1-Related Disorders, Wilson disease, Xeroderma pigmentosum, X-linked agammaglobulinemia,

X-linked hereditary motor and sensory neuropathy, X-linked severe combined immunodeficiency, and Zellweger syndrome.

[0262] Some embodiments provide methods for using the Cas9 DNA editing fusion proteins provided herein. In some embodiments, the fusion protein is used to introduce a point mutation into a nucleic acid by deaminating a target nucleobase, e.g., a C residue. In some embodiments, the deamination of the target nucleobase results in the correction of a genetic defect, e.g., in the correction of a point mutation that leads to a loss of function in a gene product. In some embodiments, the genetic defect is associated with a disease or disorder, e.g., a lysosomal storage disorder or a metabolic disease, such as, for example, type I diabetes. In some embodiments, the methods provided herein are used to introduce a deactivating point mutation into a gene or allele that encodes a gene product that is associated with a disease or disorder. For example, in some embodiments, methods are provided herein that employ a Cas9 DNA editing fusion protein to introduce a deactivating point mutation into an oncogene (e.g., in the treatment of a proliferative disease). A deactivating mutation may, in some embodiments, generate a premature stop codon in a coding sequence, which results in the expression of a truncated gene product, e.g., a truncated protein lacking the function of the full-length protein.

[0263] In some embodiments, the purpose of the methods provide herein is to restore the function of a dysfunctional gene via genome editing. The Cas9 deaminase fusion proteins provided herein can be validated for gene editing-based human therapeutics in vitro, e.g., by correcting a disease-associated mutation in human cell culture. It will be understood by the skilled artisan that the fusion proteins provided herein, e.g., the fusion proteins comprising a Cas9 domain and a nucleic acid deaminase domain can be used to correct any single point T->C or A->G mutation. In the first case, deamination of the mutant C back to U corrects the mutation, and in the latter case, deamination of the C that is base-paired with the mutant G, followed by a round of replication, corrects the mutation.

[0264] The successful correction of point mutations in disease-associated genes and alleles opens up new strategies for gene correction with applications in therapeutics and basic research. Site-specific single-base modification systems like the disclosed fusions of GeoCas9 and deaminase enzymes or domains also have applications in "reverse" gene therapy, where certain gene functions are purposely suppressed or abolished. In these cases, site-specifically mutating Trp (TGG), Gln (CAA and CAG), or Arg (CGA) residues to premature stop codons (TAA, TAG, TGA) can be used to abolish protein function in vitro, ex vivo, or in vivo.

[0265] The instant disclosure provides methods for the treatment of a subject diagnosed with a disease associated with or caused by a point mutation that can be corrected by a GeoCas9 base editing fusion protein provided herein. For example, in some embodiments, a method is provided that comprises administering to a subject having such a disease, e.g., a cancer, an effective amount of a GeoCas9 deaminase fusion protein that corrects the point mutation or introduces a deactivating mutation into the disease-associated gene. In some embodiments, the disease is a proliferative disease. In some embodiments, the disease is a genetic disease. In some embodiments, the disease is a neoplastic disease. In some embodiments, the disease is a metabolic disease. In some embodiments, the disease is a lysosomal storage disease. Other diseases that can be treated by correcting a point mutation or introducing a deactivating mutation into a disease-associated gene will be known to those of skill in the art, and the disclosure is not limited in this respect.

[0266] The instant disclosure provides methods for the treatment of additional diseases or disorders, e.g., diseases or disorders that are associated or caused by a point mutation that can be corrected by deaminase-mediated gene editing. Some such diseases are described herein, and additional suitable diseases that can be treated with the strategies and fusion proteins provided herein will be apparent to those of skill in the art based on the instant disclosure. Exemplary suitable diseases and disorders are provided herein. It will be understood that the numbering of the specific positions or residues in the respective sequences depends on the particular protein and numbering scheme used. Numbering might be different, e.g., in precursors of a mature protein and the mature protein itself, and differences in sequences from species to species may affect numbering. One of skill in the art will be able to identify the respective residue in any homologous protein and in the respective encoding nucleic acid by methods well known in the art, e.g., by sequence alignment and determination of homologous residues. Exemplary suitable diseases and disorders include, without limitation, cystic fibrosis (see, e.g., Schwank et al., Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell stem cell.* 2013; 13:653-658; and Wu et. al., Correction of a genetic disease in mouse via use of CRISPR-Cas9. *Cell stem cell.* 2013; 13:659-662, neither of which uses a deaminase fusion protein to correct the genetic defect); phenylketonuria—e.g., phenylalanine to serine mutation at position 835 (mouse) or 240 (human) or a homologous residue in phenylalanine hydroxylase gene (T>C mutation)—see, e.g., McDonald et al., *Genomics.* 1997; 39:402-405; Bernard-Soulier syndrome (BSS)—e.g., phenylalanine to serine mutation at position 55 or a homologous residue, or cysteine to arginine at residue 24 or a homologous residue in the platelet membrane glycoprotein IX (T>C mutation)—see, e.g., Noris et al., *British Journal of Haematology.* 1997; 97:312-320, and Ali et al., *Hematol.* 2014; 93:381-384; epidermolytic hyperkeratosis (EHK)—e.g., leucine to proline mutation at position 160 or 161 (if counting the initiator methionine) or a homologous residue in keratin 1 (T>C mutation)—see, e.g., Chipev et al., *Cell.* 1992; 70:821-828, see also accession number P04264 in the UNIPROT database at www [dot] uniprot [dot] org; chronic obstructive pulmonary disease (COPD)—e.g., leucine to proline mutation at position 54 or 55 (if counting the initiator methionine) or a homologous residue in the processed form of α_1-antitrypsin or residue 78 in the unprocessed form or a homologous residue (T>C mutation)—see, e.g., Poller et al., *Genomics.* 1993; 17:740-743, see also accession number P01011 in the UNIPROT database; Charcot-Marie-Toot disease type 4J—e.g., isoleucine to threonine mutation at position 41 or a homologous residue in FIG. **4** (T>C mutation)—see, e.g., Lenk et al., PLOS Genetics. 2011; 7: e1002104; neuroblastoma (NB)—e.g., leucine to proline mutation at position 197 or a homologous residue in Caspase-9 (T>C mutation)—see, e.g., Kundu et al., 3 *Biotech.* 2013, 3:225-234; von Willebrand disease (vWD)—e.g., cysteine to arginine mutation at position 509 or a homologous residue in the processed form of von Willebrand factor, or at position 1272 or a homologous residue in the unpro-

cessed form of von Willebrand factor (T>C mutation)—see, e.g., Lavergne et al., *Br. J. Haematol.* 1992, see also accession number P04275 in the UNIPROT database; 82:66-72; myotonia congenital—e.g., cysteine to arginine mutation at position 277 or a homologous residue in the muscle chloride channel gene CLCN1 (T>C mutation)—see, e.g., Weinberger et al., *The J. of Physiology.* 2012; 590:3449-3464; hereditary renal amyloidosis—e.g., stop codon to arginine mutation at position 78 or a homologous residue in the processed form of apolipoprotein AII or at position 101 or a homologous residue in the unprocessed form (T>C mutation)—see, e.g., Yazaki et al., *Kidney Int.* 2003; 64:11-16; dilated cardiomyopathy (DCM)—e.g., tryptophan to Arginine mutation at position 148 or a homologous residue in the FOXD4 gene (T>C mutation), see, e.g., Minoretti et. al., *Int. J. of Mol. Med.* 2007; 19:369-372; hereditary lymphedema—e.g., histidine to arginine mutation at position 1035 or a homologous residue in VEGFR3 tyrosine kinase (A>G mutation), see, e.g., Irrthum et al., *Am. J. Hum. Genet.* 2000; 67:295-301; familial Alzheimer's disease—e.g., isoleucine to valine mutation at position 143 or a homologous residue in presenilin1 (A>G mutation), see, e.g., Gallo et. al., *J. Alzheimer's disease.* 2011; 25:425-431; Prion disease—e.g., methionine to valine mutation at position 129 or a homologous residue in prion protein (A>G mutation)—see, e.g., Lewis et. al., *J. of General Virology.* 2006; 87:2443-2449; chronic infantile neurologic cutaneous articular syndrome (CINCA)—e.g., Tyrosine to Cysteine mutation at position 570 or a homologous residue in cryopyrin (A>G mutation)—see, e.g., Fujisawa et. al. Blood. 2007; 109:2903-2911; and desmin-related myopathy (DRM)—e.g., arginine to glycine mutation at position 120 or a homologous residue in aß crystallin (A>G mutation)—see, e.g., Kumar et al., *J. Biol. Chem.* 1999; 274:24137-24141. The entire contents of all references and database entries is incorporated herein by reference.

[0267] Additional exemplary genes in the human genome that may be targeted by the base editors or complexes of this disclosure are provided herein in Tables 1 and 2. Table 1 includes gene mutations that may be correcteded by changing a cytosine (C) to a thymine (T), for example, using a GeoBE3 or GeoBE4 nucleobase editor. Table 2 includes gene mutations that may be corrected by changing a guanine (G) to an adenine (A), for example, using a GeoBE3 or GeoBE4. Identification of exemplary genes that may be targeted using any of the base editors provided herein were identified using the ClinVar database. The 'gRNA' tab shows the potential target sequence (followed by the PAM, which would not be included in the gRNA). In Table 1, which list targets for C to T changes, the target base is indicated by 'Y' (the corresponding gRNA would have a C here). In Table 2, which lists targets for G to A changes the target base is indicated as 'R' (the corresponding gRNA would have a G here). To account for a potential large window of editing for GeoBEs, include are diseases whose mutated base can be placed within position 2-12 of the window (PAM is in position 21-23). The PAM for GeoCas9 has been set to NNNNCRAA (SEQ ID NO: 79).

TABLE 1

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_000071.2(CBS): c.833T > C (p.Ile278Thr)	5742905	CBS	['TCTGCAGATCAYTGG GGTGGATCCCGAA']	['Homocystinuria due to CBS deficiency', 'Homocystinuria, pyridoxine-responsive', 'not provided']	82
NM_000521.3(HEXB): c.185C > T (p.Ser62Leu)	820878	HEXB	['TGCCGCTCTYGGTGA AGATGACCCCGAA']	['Sandhoff disease, infantile type']	83
NM_139058.2(ARX): c.98T > C (p.Leu33Pro)	28936077	ARX	['CATCCYGGGCCGGA GGAGCCCGTGCAAA']	['Mental retardation, with or without seizures, ARX-related, X-linked']	84
NM_024960.4(PANK2): c.437T > C (p.Met146Thr)	28939088	PANK2	['AYGGCATCTCGTGG AGATAGCACCAAA', 'AAYGGCATCTCGTGG AGATAGCACCAAA']	['Hypoprebetalipoprotein emia, acanthocytosis, retinitis pigmentosa, and pallidal degeneration']	85, 86
NM_000045.3(ARG1: c.32T > C (p.Ile11Thr)	28941474	ARG1	['TAGGGATTAYTGGA GCTCCTTTCTC AAA']; ['TAGGGATTAYTGGA GCTCCTTTCTCAAA']	['Arginase deficiency']	87, 87
NM_024426.4(WT1): c.1351T > C (p.Phe451Leu)	28941777	WT1	['CCAYTCCAGTGTAAA ACTTGTCAGCGAA']	['Diffuse mesangial sclerosis']	88
NM_000559.2(HBG1): c.-251T > C	35710727	HBG1	['CCCCTYCCCCACACT ATCTCAATGCAAA']	['Fetal hemoglobin quantitative trait locus 1']	89

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_000518.4(HBB): c.344T > C (p.Leu115Pro)	36015961	HBB	['CTGTGTGCYGGCCCA TCACTTTGGCAAA']	['Beta thalassemia intermedia']	90
m.3394T > C	41460449	MT-ND1	['AATTCTAGGCYATAT ACAACTACGCAAA']	['Leber optic atrophy']	91
NM_000548.3(TSC2): c.3106T > C (p.Ser1036Pro)	45517281	TSC2	['TCTTCYCCAACTTCA CGGCTGTCCCGAA']	['Tuberous sclerosis syndrome', 'Tuberous sclerosis 2']	92
NM_024514.4(C YP2R1): c.296T > C (p.Leu99Pro)	61495246	CYP2R1	['AAAGGAATGCCYTG TTCATCAAAGCGAA']	['Vitamin d hydroxylation-deficient rickets, type lb']	93
NM_002618.3(PEX13): c977T > C (p.Ile326Thr)	61752115	PEX13	['AGGACTTAYACCTGC GAATTATGTCAAA']	['Peroxisome biogenesis disorder 11B']	94
NM_014165.3(NDUFAF 4):c.194T > C (p.Leu65Pro)	63751061	NDUFAF4	['CYAAAAGATGTGTA TGTTGATTCCAAA', 'TCYAAAAGATGTGTA TGTTGATTCCAAA']; ['CYAAAAGATGTGTA TGTTGATTCCAAA']	['Mitochondrial complex I deficiency']	97, 96, 97
NM_000104.3(CYP1B1): c.2T > C (p.Met1Thr)	72549389	CYP1B1	['CCAGCAYGGGCACC AGCCTCAGCCCGAA']	['Irido-corneo-trabecular dysgenesis']	98
NM_000531.5(OTC): c.386 + 2T > C	72554359	OTC	['GYTTGTAAATATTTT CTTCTCTCCAAA', 'GGYTTGTAAATATTT TCTTCTCTCCAAA']; ['GYTTGTAAATATTTT CTTCTCTCCAAA', 'GGYTTGTAAATATTT TCTTCTCTCCAAA']	['not provided']	99, 100, 99, 100
NM_000098.2(CPT2): c.1342T > C (p.Phe448Leu)	74315297	CPT2	['CTGCGTCCAGYTTCA GAGAGGAGGCAAA']	['CARNITINE PALMITOYLTRANSFE RASE II DEFICIENCY, LATE-ONSET', 'not provided']	101
NM_007375.3(TARDBP): c.*83T > C	80356744	TARDBP	['AAATACAYATGTACT AAGAATTTTCAAA']	['Amyotrophic lateral sclerosis type 10']	102
NM_000271.4(NPC1): c.3182T > C(p.Ile1061Thr)	80358259	NPC1	['CCGACTTAYAGCCA GTAATGTCACCGAA']	['Niemann-Pick disease type C1']	103
NM_198159.2(MITF): c.1051T > C(p.Ser351Pro)	104893744	MITF	['TTAAAAGCAYCCGT GGACTATATCCGAA']	['Waardenburg syndrome type 2A']	104
NM_001018077.1(NR3C1): c.1712T > C (p.Val571Ala)	104893911	NR3C1	['AAGYGATTGCAGCA GTGAAATGGGCAAA']	['Pseudohermaphroditism, female, with hypokalemia, due to glucocorticoid resistance']	105
NM_004577.3(PSPH): c.155T > C (p.Met52Thr)	104894036	PSPH	['AGCCAYGGGCGGGG CAGTGCCTTTCAAA']	['Deficiency of phosphoserine phosphatase']	106
NM_020661.2(AICDA): c.452T > C (p.Phe151Ser)	104894327	AICDA	['CTGGAATACTTYTGT AGAAAACCACGAA']	['Immunodeficiency with hyper IgM type 2']	107
NM_000432.3(MYL2): c.52T > C (p.Phe18Leu)	104894370	MYL2	['GTGYTCTCCATGTTC GAACAGACCCAAA']	['Familial hypertrophic cardiomyopathy 10']	108

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_000371.3(TTR): c.191T > C (p.Phe64Ser)	104894665	TTR	['GCCATYTGCCTCTGG GTAAGTTGCCAAA']	['Amyloidogenic transthyretin amyloidosis', 'AMYLOIDOSIS, LEPTOMENINGEAL, TRANSTHYRETIN-RELATED']	109
NM_033290.3(MID1): c.884T > C (p.Leu295Pro)	104894866	MID1	['CYGGCTCAGCAGATT GCAAACTGCAAA', 'ACYGGCTCAGCAGAT TGCAAACTGCAAA', 'TTCGCAAACYGGCTC AGCAGATTGCAAA']	['Opitz-Frias syndrome']	110, 111, 112
NM_001109878.1(TBX22): c.641T > C (p.Leu214Pro)	104894946	TBX22	['CATTCYGCAATCCAT GCATAAGTACAAA']	['Cleft palate with ankyloglossia']	113
NM_001065.3(TNFRSF1A): c.175T > C (p.Cys59Arg)	104895217	TNFRSF1A	['TTCGATTTGCYGTAC CAAGTGCCACAAA']	['TNF receptor-associated periodic fever syndrome (TRAPS)']	114
NM_000441.1 (SLC26A4): c, 1588T > C (p.Tyr530His)	111033254	SLC26A4	['TATCYACAAAAGTA CCAAGAATTACAAA']	['Pendred syndrome', 'Enlarged vestibular aqueduct syndrome']	115
NM_206933.2(USH2A): c.10561T > C (p.Trp3521Arg)	111033264	USH2A	['TAAACYGGAGAAAA CCTATACAATCAAA']	['Usher syndrome, type 2A']	116
NM_006364.2(SEC23A): c.H44T > C (p.Phe382Leu)	118204000	SEC23A	['ACTTCCTTAYTCAAA CAAACTTTTCAAA']	['Craniolenticulo sutural dysplasia']	117
NM_000018.3(ACADV L):c.1372T > C (p.Phe458Leu)	118204017	ACADVL	['GCATCYTCCGGATCT TTGAGGGGACAAA']	['Very long chain acyl-CoA dehydrogenase deficiency']	118
NM_000123.3(ERCC5): c.2573T > C (p.Leu858Pro)	121434575	—	['TTATTTGCYTGGAAG TGATTATACCGAA']	['Xeroderma pigmentosum, group G']	119
NM_000280.4(PAX6): c.773T > C (p.Phe258Ser)	121907925	PAX6	['GGTATGGTYTTCTAA TCGAAGGGCCAAA']	['Congenital ocular coloboma', 'Coloboma of optic disc']	120
NM_005857.4(ZMPSTE24): c.1018T > C (p.Trp340Arg)	121908093	ZMPSTE24	['GCACYGGAAGTTGG GACATACAGTCAAA']	['Mandibulo acral dysplasia with type B lipodystrophy', 'not provided']	121
NM_031433.3(MFRP): c.545T > C (p.Ile182Thr)	121908190	—	['AGACCATGCAAYAC AGCTCAAGATCGAA']	['Nanophthalmos 2']	122
NM_133459.3(CCBE1): c.520T > C (p.Cys174Arg)	121908254	CCBE1	['TGGGAAGACAYGTA CCAGGGGAGACAAA']	['Hennekam lymphangiectasia-lymphedema syndrome']	123
NM_014845.5(FIG4): c.122T > C (p.Ile41Thr)	121908287	FIG4	['TCTTGAAGAYTGATA GAACAGAACCAAA']	['Charcot-Marie-Tooth disease, type 4J', 'not provided']	124
NM_003835.3(RGS9): c.895T > C (p.Trp299Arg)	121908449	RGS9	['GGAACGAYGGGCCT TCAACTTCAGCGAA']	['Prolonged electroretinal response suppression']	125

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_000492.3(CFTR): c.3857T > C (p.Phe1286Ser)	121909028	CFTR	['TYTGGAGTGATACCA CAGGTGAGCAAA', 'CTYTGGAGTGATACC ACAGGTGAGCAAA']; ['TYTGGAGTGATACCA CAGGTGAGCAAA', 'CTYTGGAGTGATACC ACAGGTGAGCAAA']	['Cystic fibrosis']	126, 128, 127, 128
NM_005570.3(LMAN1): c.2T > C (p.Met1Thr)	121909253	LMAN1	['GAATCCAAGAYGGC GGGATCCAGGCAAA']	['Combined deficiency of factor V and factor VIII, 1']	129
NM_006302.2(MOGS): c.1954T > C (p.Phe652Leu)	121909292	MOGS	['CYTTGCAGACTTTGG GAACCACACAAA', 'TCYTTGCAGACTTTG GGAACCACACAAA']	['Congenital disorder of glycosylation type 2B']	130, 131
NM_002427.3(MMP13): c.224T > C (p.Phe75Ser)	121909497	MMP13	['TTTCTYCGGCTTAGA GGTGACTGGCAAA']	['Spondyloepimetaphysea 1 dysplasia, Missouri type']	132
NM_002427.3(MMP13): c.221T > C (p.Phe74Ser)	121909498	MMP13	['TYCTTCGGCTTAGAG GTGACTGGCAAA', 'TTYCTTCGGCTTAGA GGTGACTGGCAAA']	[]	133, 134
NM_000488.3(SERPINC1): c.667T > C (p.Ser223Pro)	121909572	SERPINC1	['TGGGTGYCCAATAA GACCGAAGGCCGAA']	['Antithrombin III deficiency']	135
NM_001173464.1(KIF21A):c.3029T > C (p.Ile1010Thr)	121912587	KIF21A	['CCAACAYAATGCAG ATGGAAGAAGCAAA']	['Fibrosis of extraocular muscles, congenital, 1']	136
NM_000257.3(MYH7): c.5378T > C (p.Leu1793Pro)	121913654	MYH7	['TAAGGACCYGCAGC ACCGGCTGGACGAA']	['Familial hypertrophic cardiomyopathy 1', 'Myosin storage myopathy', 'Left ventricular noncompaction 5', 'Cardiomyopathy']	137
NM_000124.3(ERCC6): c.2960T > C (p.Leu987Pro)	121917905	ERCC6	['AATAGAGTGCYAAA AGACCCAAAACAAA']	['Cerebro-oculo-facio-skeletal syndrome']	138
NM_000371.3(TTR): c.88T > C (p.Cys30Arg)	121918083	TTR	['TGAATCCAAGYGTCC TCTGATGGTCAAA']	['Amyloidogenic transthyretin amyloidosis', 'Cardiomyopathy']	139
NM_000371.3(TTR): c.272T > C (p.Val91Ala)	121918084	TTR	['GATATACAAAGYGG AAATAGACACCAAA']	['Amyloidogenic transthyretin amyloidosis']	140
NM_000371.3(TTR): 5c.20T > C (p.Phe84Leu)	121918091	TTR	['GGAGGAAYTTGTAG AAGGGATATAC AAA']; ['GGAGGAAYTTGTAG AAGGGATATACAAA']	['Amyloidogenic transthyretin amyloidosis']	141, 141
NM_000371.3(TTR): c.265T > C (p.Tyr89His)	121918100	TTR	['GATAYACAAAGTGG AAATAGACACCAAA']	['AMYLOIDOSIS, LEPTOMENINGEAL, TRANSTHYRETIN-RELATED']	143

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_000322.4(PRPH2): c.554T > C (p.Leu185Pro)	121918563	PRPH2	['CYGGACTTTTCCTCC AAAGAAGTCAAA', 'CCYGGACTTTTCCTCC AAAGAAGTCAAA', 'CAATCGCTACCYGGA CTTTTCCTCCAAA']	['Patterned dystrophy of retinal pigment epithelium', 'Retinitis pigmentosa 7, digenic', 'not provided', 'Leber congenital amaurosis 18']	144, 145, 146
NM_001035.2(RYR2): c.1298T > C (p.Leu433Pro)	121918602	RYR2	['GCCYTGATGCTCTCA GCAAGAAAGCGAA']	['Arrhythmogenic right ventricular cardiomyopathy, type 2', 'Catecholaminergic polymorphic ventricular tachycardia', 'Long QT syndrome']	147
NM_001159287.1(TPI1): c.832T > C (p.Phe278Leu)	121964847	TPI1	['CGAAYTCGTGGACA TCATCAATGCCAAA']	['Triosephosphate isomerase deficiency']	148
NM_000398.6(CYB5R3): c.446T > C (p.Leu149Pro)	121965008	CYB5R3	['CAGTGGGCTGCYGG TCTACCAGGGCAAA']	['METHEMOGLOBINE MIA, TYPE 1']	149
NM_004586.2(RPS6KA3): c.803T > C (p.Phe268Ser)	122454131	RPS6KA3	['ACACTCCCTTYCCAA GGAAAAGATCGAA']	['Coffin-Lowry syndrome']	150
NM_001128834.2(PLP1): c.671T > C (p.Leu224Pro)	132630283	PLP1	['TGGCTCCAACCYTCT GTCCATCTGCAAA']	['Peliz aeus-Merzb acher disease']	151
NM_000133.3(F9): c.1357T > C (p.Trp453Arg)	137852269	F9	['ATGTCAACYGGATTA AGGAAAAAACAAA']	['Hereditary factor IX deficiency disease']	152
NM_000044.3(AR): c.2596T > C (p.Ser866Pro)	137852597	AR	['CCTGGACYCCGTGCA GCCTGTAAGCAAA']	['Androgen resistance syndrome']	153
NM_001171507.2(MCFD2): c.407T > C (p.Ile136Thr)	137852914	MCFD2	['GATACAYTGACTATG CTGAATTTGCAAA']	['Factor v and factor viii, combined deficiency of, 2']	154
NM_001001486.1 (ATP2C1): c.1751T > C (p.Leu584Pro)	137853015	ATP2C1	['AGCCAGTCGTCYGG GATTGTATTCCAAA']	['Familial benign pemphigus']	155
NM_001127695.1(CTSA): c.707T > C (p.Leu236Pro)	137854546	CTSA	['TCTCYCCAGACCCAC TGCTGCTCTCAAA']	['Galactosialidosis, early infantile']	156
NM_000371.3(TTR): c.190T > C (p.Phe64Leu)	138065384	TTR	['GCCAYTTGCCTCTGG GTAAGTTGCCAAA']	['Cardiomyopathy', 'not specified']	157
NM_004614.4(TK2): c.173A > G (p.Asn58Ser)	138439950	TK2	['ATAYTGCCCTCGACA CAGATCTGGCAAA']	['Mitochondrial DNA depletion syndrome 2']	158
NM_020320.3(RARS2): c.35A > G (p.Gln12Arg)	147391618	RARS2	['CATACCYGGCAAGC AATAGCGCGGCGAA']	['Pontocerebellar hypoplasia type 6']	159
NM_206933.2(USH2A): c.12295-2A > G	151148854	USH2A	['TGTATGTCYACAGAA GGACAGAAGCAAA']	['Usher syndrome, type 2A']	160
NM_000080.3(CHRNE): c.223T > C (p.Trp75Arg)	193919341	—	['AGCGTCYGGATTGG AATCGTGAGTCAAA']	['MYASTHENIC SYNDROME, CONGENITAL, 4B, FAST-CHANNEL']	161

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_004329.2(BMPR1A): c.1409T > C (p.Met470Thr)	199476089	BMPR1A	['AGATAYGCGTGAGG TTGTGTGTGTCAAA']	['Juvenile polyposis syndrome']	162
NM_006214.3(PHYH): c.135-2A > G	201578674	PHYH	['ATACCYAAAGGAGA AAAAGAATCCCAAA']	['Refsum disease, adult, 1']	163
NM_013382.5(POMT2): c.2242T > C (p.Trp748Arg)	267606964	POMT2	['CTCAYGGGACTTTTG AGGCCACTGCAAA']	['Congenital muscular dystrophy-dystroglycanopathy with mental retardation, type B2']	164
NM_000084.4(CLCN5): c.674T > C (p.Leu225Pro)	273585645	CLCN5	['GAACATCCYGTGCC ACTGCTTCAACAAA']	['Dent disease 1']	165
NM_005211.3(CSF1R): c.2624T > C (p.Met875Thr)	281860279	CSF1R	['AAAYGGCCCAGCCT GCATTTGCCCCAAA']	['Hereditary diffuse leukoencephalopathy with spheroids']	166
NM_004614.4(TK2): c.644T > C (p.Leu215Pro)	281865497	TK2	['CCATCYCCATGAGG AGTGGCTCATCAAA']	['Mitochondrial DNA depletion syndrome 2']	167
NM_000155.3(GALT): c.336T > C (p.Ser112=)	367543254	GALT	['CCAGYGATCATCCCC TTTTCCAAGCAAA']	['Deficiency of UDPglucose-hexose-1-phosphate uridylyltransferase']	168
NM_022445.3(TPK1): c.656A > G (p.Asn219Ser)	371271054	TPK1	['AYTGGAAGTACTGA CCAATGTTCCAAA', 'TAYTGGAAGTACTGA CCAATGTTCCAAA']	['THIAMINE METABOLISM DYSFUNCTION SYNDROME 5 (EPISODIC ENCEPHALOPATHY TYPE)']	169, 170
NM_001282227.1 (CECR1): c.1232A > G (p.Tyr411Cys)	376785840	CECR1	['AATCAYAGGACAAG CCTTTGGCACCAAA']	['Polyarteritis nodosa']	171
NM_004453.3(ETFDH): c.1001T > C (p.Leu334Pro)	377686388	ETFDH	['TACCYGAGTCCATTT AGAGAGTTCCAAA']	['Glutaric aciduria, type 2']	172
NM_002465.3(MYBPC1): c.706T > C (p.Trp236Arg)	387906657	MYBPC1	['ACGTAYGGGAGTTG CTGAAGAACGCGAA']	['Distal arthrogryposis type IB']	173
NM_001256714.1(DNAAF3): c.386T > C (p.Leu129Pro)	387907151	DNAAF3	['ACCTGCYGCGGACC CTGTCCCGAGCGAA']	['Kartagener syndrome', 'Ciliary dyskinesia, primary, 2']	174
NM_152296.4(ATP1A3): c.2431T > C (p.Ser811Pro)	387907282	ATP1A3	['CATCYCACTGGCGTA CGAGGCTGCCGAA']	['Alternating hemiplegia of childhood 2']	175
NM_000344.3(SMN1): c.388T > C (p.Tyr130His)	397514518	SMN1	['ACTGGAYATGGAAA TAGAGAGGAGCAAA']	['Kugelberg-Welander disease']	176
NM_024531.4(SLC52A2): c.368T > C (p.Leu123Pro)	397514538	SLC52A2	['TGCTGGCACYGGCAT GCTGTGCCTCGAA']	['Brown-Vialetto-Van Laere syndrome 2']	177
NM_001006657.1(WDR35): c.1592T > C (p.Leu531Pro)	397515533	WDR35	['AGTCYACCTAATGTT GGTTTGATTCAAA']	['Cranioectodermal dysplasia 2']	178

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_004595.4(SMS): c.449T > C (p.Ile150Thr)	397515552	SMS	['ATCAAAATAYAAAA ATTCTACACTCGAA']	['Snyder Robinson syndrome']	179
NM_206933.2(USH2A): c.5857 + 2T > C	397518022		['CAGGAGCAGGYAAA TACTTATCTTCAAA']	['Usher syndrome, type 2A']	180
NM_000212.2(ITGB3): c.2231T > C (p.Leu744Pro)	398122374	—	['AAACTCCYCATCACC ATCCACGACCGAA']	['Platelet-type bleeding disorder 16']	181
NM_000019.3(ACAT1): c.730 + 2T > C	398123096	ACAT1	['CAGTAAAAGGYAGA GATAATGTTCCAAA']; ['CAGTAAAAGGYAGA GATAATGTTCCAAA']	['Deficiency of acetyl-CoA acetyltransferase', 'not provided']	182, 182
NM_000169.2(GLA): c.899T > C (p.Leu300Pro)	398123223	—	['CYCCGACACATCAG CCCTCAAGCCAAA', 'CCYCCGACACATCAG CCCTCAAGCCAAA']	['Fabry disease']	183, 184
NM_014795.3(ZEB2): c.73 + 2T > C	398124282	ZEB2	['AAAACGGYAAGAAG CAGCCCGAACCAAA']	['Mowat-Wilson syndrome']	185
NM_000353.2(TAT): c.236-5A > G	587776512	TAT	['TCCCCTTTYTATGGG AGGAAAACACAAA']	['Tyrosinemia type 2']	186
NM_001142519.1(FAM111A): c.1531T > C (p.Tyr511His)	587777012	FAM111A	['CCAGAGYATGTCCAT ATGTATACTCAAA']	['Kenny-Caffey syndrome type 2']	187
NM_052844.3(WDR34): c.1307A > G (p.Lys436Arg)	587777098	WDR34	['CAGATACYTGAGGG AGAGCTGCAGCGAA']	['Short-rib thoracic dysplasia 11 with or without polydactyly']	188
NM_014740.3(EIF4A3): c.809A > G (p.Asp270Gly)	587777204	EIF4A3	['TGTCGTAGAGGYCA CACAGAGTGTCAAA']	['Richieri Costa Pereira syndrome']	189
NM_012338.3(TSPAN12): c.413A > G (p.Tyr138Cys)	587777283	TSPAN12	['TCCAYAATTTGTCAT CCTGGCTTTCAAA']	['Exudative vitreoretinopathy 5']	190
NM_001159287.1(TPI1): c.833T > C (p.Phe278Ser)	587777440	TPI1	['CGAATYCGTGGACA TCATCAATGCCAAA']	['Triosephosphate isomerase deficiency']	191
NM_017696.2(MCM9): c.1732 + 2T > C	587777871	MCM9	['TAGCAGAAGGYCTA TTTCATTCAGCGAA']	['Premature ovarian failure 1', 'Ovarian dysgenesis 4']	192
NM_178151.2(DCX): c.641T > C (p.Ile214Thr)	587783574	DCX	['CCTCACTGATAYCAC AGAAGCCATCAAA']	['Heterotopia']	193
NM_022455.4(NSD1): c.5989T > C (p.Tyr1997His)	587784171	NSD1	['TAATTTCYATATGCT CACCCTAGACAAA']	['Sotos syndrome 1']	194
NM_017653.3(DYM): c.621-2A > G	775414124	DYM	['CYATACAAAAAGGA AAAAAAAATCAAA', 'ACYATACAAAAAGGA AAAAAAAATCAAA']	['Dyggve-Melchior-Clausen syndrome']	195, 196
NM_001987.4(ETV6): c.1046T > C (p.Leu349Pro)	786205155	ETV6	['TCAGTTGCYTTCTGA CAGCCGGTACGAA']	['Thrombocytopenia', 'LEUKEMIA, ACUTE LYMPHOBLASTIC; ALL']	197

TABLE 1-continued

Human gene mutations that may be corrected by changing a cytosine (C) to a thymine (T) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a C, indicated by a "Y". The "gRNAs" sequences correspond to SEQ ID NOs: 82-199. It should be appreciated that each instance of thymine "T" in the gRNAs listed in the below tables is uracil "U", as uracil occurs in RNA.

Name (dbSNP)	RS# Symbol	Gene	gRNAs	Phenotypes	SEQ ID NO(s):
NM_004370.5(COL12A1): c.7001T > C (p.Ile2334Thr)	796052093	COL12A1	['GAGCAYTGGGGACG ATAATTTTAACAAA']	['BETHLEM MYOPATHY 2']	198
NM_014191.3(SCN8A): c.4889T > C (p.Leu1630Pro)	796053222	SCN8A	['CATCTTGCGTCYGAT CAAAGGCGCCAAA']	['not provided']	199

TABLE 2

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.
SEQ ID NO(s):

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_000487.5(ARSA): c.*96A > G	6151429	ARSA	['TTTGTGCCTGATAAC GTARTAACACC AG']; ['TTTGTGCCTGATAAC GTARTAACACCAG']	['Metachromatic leukodystrophy', 'Arylsulfatase A pseudodeficiency', 'not provided']	200, 201
NM_000060.3(BTD): c.755A > G (p.Asp252Gly)	28934601	BTD	['TTTGATATATTGTTC TTTGRCCCTGCCA']	['Biotinidase deficiency']	202
NM_005263.3(GFI1): c.1208A > G (p.Lys403Arg)	28936382	GFI1	['TTCGGCTGCGACCTC TGTGGGARGGGTT']	['Neutropenia, nonimmune chronic idiopathic, of adults']	203
NM_000527.4(LDLR): c.2483A > G (p.Tyr828Cys)	28942085	LDLR	['TTTGACAACCCCGTC TRTCAGAAGACCA']	['Familial hypercholesterolemia', 'not provided']	204
NM_000060.3(BTD): c.880A > G (p.Ile294Val)	35976361	BTD	['TTTGCTGTTGCCTTT GGCRTCAACGTTC']	['Biotinidase deficiency']	205
NM_000249.3(MLH1): c.544A > G (p.Arg182Gly)	63750211	MLH1	['TTTGGAAGTTGTTGG CRGGTACAGTCCA']	['Hereditary Nonpolyposis Colorectal Neoplasms']	206
NM_000484.3(APP): c.2078A > G (p.Glu693Gly)	63751039	APP	['TTTGTTTTCAAGGTG TTCTTTGCAGRAG']	['Alzheimer disease', 'Alzheimer disease, type 1', 'Cerebral amyloid angiopathy, APP-related', 'not provided']	207
NM_000249.3(MLH1): c.122A > G (p.Asp41Gly)	63751094	MLH1	['TTTGATTTGCCAGTT TAGRTGCAAAATC']	['Hereditary Nonpolyposis Colorectal Neoplasms']	208
NM_000463.2(UGTlA1): c.992A > G (p.Gln331Arg)	72551348	—	['TTTGGGCAAAATCCC TCRGACAGTAAGA']	['Crigler-Najjar syndrome, type 11']	209
NM_000531.5(GTC): c.238A > G (p.Lys80Glu)	72554332	OTC	['TTTGCCTTTATTGCA AGGGRAGTCCTTA']	['Ornithine carbamoyltransferase deficiency', 'not provided']	210

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_000531.5(OTC): c.277A > G (p.Thr93Ala)	72554344	OTC	['TTTGAGAAAAGAAG TACTCGARCAAGAT']	['not provided']	211
NM_000404.2(GLB 1): c.1498A > G (p.Thr500Ala)	72555368	GLB1	['TTTGCAGGGTTTGGT TTCTAACCTGRCT', 'TTTGGTTTCTAACCTG RCTCTCAGTTCC']	['Mucopolysaccharidosis, MPS-IV-B']	212, 213
NM_170784.2(MKKS): c.169A > G (p.Thr57Ala)	74315399	MKKS	['TTTGGAGGTTACGTG TGTACARCCTCAC']	['Bardet-Biedl syndrome 6']	214
NM_000311.3(PRNP): c.547A > G (p.Thr183Ala)	74315411	PRNP	['TTTGTGCACGACTGC GTCAATATCRCAA']; ['TTTGTGCACGACTGC GTCAATATCRCAA']	['Genetic prion diseases', 'Spongiform encephalopathy with neurop sy chi atric features']	215, 216
NM_000492.3(CFTR): c.3140-26A > G	76151804	CFTR	['TTTGTGTTTATGTTA TTTGCARTGTTTT']	['Cystic fibrosis']	217
NM_000303.2(PMM2): c.563A > G (p.Asp188Gly)	80338704	PMM2	['TTTGATGTCTTTCCT GATGGATGGGRCA']	['Carbohydrate-deficient glycoprotein syndrome type F, 'not provided']	218
NM_206933.2(USH2A): c.14020A > G (p.Arg4674Gly)	80338904	USH2A	['TTTGTATTACGAATT ATACRGAAGACAA']	['Retinitis pigmentosa', 'Retinitis pigmentosa 39']	219
NM_000059.3(BRCA2): c.9118-2A > G	81002862	BRCA2	['TTTGTTTTGTTTTCTG TRGGTTTCAGAT']	['Familial cancer of breast', 'Breast-ovarian cancer, familial 2']	220
NM_000495.4(COL4A5): c.2746A > G (p.Ser916Gly)	104886193	COL4A5	['TTTGGGAATTCCTGG CAGGRGTGGTGTA']	['Alport syndrome, X-linked recessive']	221
NM_000495.4(COL4A5): c.2042-18A > G	104886341	COL4A5	['TTTGAACGTTTTCCT TTCAATARCTGCT']	['Alport syndrome, X-linked recessive']	222
NM_000073.2(CD3G): c.1A > G (p.Met1Val)	104894199	CD3G	['TTTGCCGGAGGACA GAGACTGACRTGGA']	['Immunodeficiency 17']	223
NM_000317.2(PTS): c.139A > G (p.Asn47Asp)	104894278	PTS	['TTTGGGAAATGCAAC AATCCARATGGCC']	['Hyperphenylalaninemia, bh4-deficient, a, due to partial pts deficiency']	224
NM_000448.2(RAG1): c.1286A > G (p.Asp429Gly)	104894292	RAG1	['TTTGCTGACAAAGAA GAAGGTGGAGRTG']	['Histiocytic medullary reticulosis']	225
NM_014239.3(EIF2B2): c.638A > G (p.Glu213Gly)	104894425	EIF2B2	['TTTGTCCAAAGCAGG TATTGRGACAACT']	['Leukoencephalopathy with vanishing white matter', 'Ovarioleukodystrophy']	226
NM_024006.5(VKORC1): c, 172A > G (p.Arg58Gly)	104894541	VKORC1	['TTCGCGCGTCTTCTC CTCCRGGTGTGCA']	['Warfarin response']	227
NM_001128085.1 (ASPA): c.71A > G (p.Glu24Gly)	104894551	—	['TTTGGAGGAACCCAT GGGAATGRGCTAA']	['Spongy degeneration of central nervous system']	228
NM_002769.4(PRSS1): c.68A > G (p.Lys23Arg)	111033567	—	['TTTGATGATGATGAC ARGATCGTTGGGG']	['Hereditary pancre-atitis']	229

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_000155.3(GALT): c.574A > G (p.Ser192Gly)	111033830	GALT	['TTTGCCCCTTGACAG GTATGGGCCRGCA']	['Deficiency of UDPglucose-hexose-1-phosphate uridylyltransferase']	230
NM_172107.2(KCNQ2): c.1764-2A > G	118192238	KCNQ2	['TTTGGGGTCTCTGTT CCCGGTRGAGTGG']	['Benign familial neonatal seizures 1']	231
NM_004519.3(KCNQ3): c914A > G (p.Asp305Gly)	118192248	KCNQ3	['TTTGAGACCTATGCA GRTGCCCTGTGGT']	['Benign familial neonatal seizures 2']	232
NM_000785.3(CYP27B1): c.566A > G (p.Glu189Gly)	118204012	CYP27B1	['TTCGGGACGTGGCG GGGGRATTTTACAA']	['Vitamin D-dependent rickets, type 1']	233
NM_015702.2(MMADHC): c.746A > G (p.Tyr249Cys)	118204046	MMADHC	['TTTGAAACTGATGAA CGCTRCCGACATT']	['Homocystinuria, cblD type, variant 1']	234
NM_014874.3(MFN2): c.827A > G (p.Gln276Arg)	119103264	MFN2	['TTTGGGCTCCAGGTG CGGCGGCRGCACA']	['Hereditary motor and sensory neuropathy with optic atrophy']	235
NM_020247.4(ADCK3): c.1541A > G (p.Tyr514Cys)	119468008	ADCK3	['TTTGGGGCAACGCG GGAATRTGACAGAT']	['Coenzyme Q10 deficiency, primary, 4']	236
NM_000197.1(HSD17B3): c.389A > G (p.Asn130Ser)	119481079	HSD17B3	['TTTGCTTCTTTCTGCC AGTCARCAATGT']	['Testosterone 17-beta-dehydrogenase deficiency']	237
NM_017780.3(CHD7): c.164A > G (p.His55Arg)	121434345	CHD7	['TTTGCCTCTTTACAG CCATCCCTTCRTC']; ['TTTGCCTCTTTACAG CCATCCCTTCRTC']	['Kallmann syndrome 5']	238, 239
NM_002894.2(RBBP8): c.1009A > G (p.Lys337Glu)	121434388	RBBP8	['TTTGGAGCTACCTCT AGTATCRAAAGTG']	['Carcinoma of pancreas']	240
NM_000430.3(PAFAH1B1): c.446A > G (p.His 149 Arg)	121434482	PAFAH1 B1	['TTTGAACGAACTCTT AAAGGACRTACAG']	['Lissencephaly 1']	241
NM_024301.4(FKRP): c.1387A > G (p.AsnAsp)	121908110	FKRP	['TTCGTGGCGCAGGCG CCTRACAACTACC']; ['TTCGTGGCGCAGGCG CCTRACAACTACC']	['Congenital muscular dystrophy-dystroglycanopathy (with or without mental retardation) type B5', 'Limb-girdle muscular dystrophy-dystroglycanopathy, type C5', 'Muscular dystrophy', 'Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A5', 'Congenital muscular dystrophy-dystroglycanopathy without mental retardation, type B5', 'not provided']	242, 243
NM_014946.3(SPAST): c.1157A > G (p.Asn386Ser)	121908514	SPAST	['TTTGGTCCACCTGGG ARTGGGAAGACAA']	['Spastic paraplegia 4, autosomal dominant']	244

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_006892.3(DNMT3B): c.2450A > G (p.Asp817Gly)	121908939	DNMT3B	['TTTGGCTTTCCTGTG CACTACACAGRCG']	['Centromeric instability of chromosomes 1, 9 and 16 and immunodeficiency']	245
NM_001130978.1(DYSF): c.5264A > G (p.Glu1755Gly)	121908961	DYSF	['TTTGTCTTCTCTCTG GGGCAGRGGCTGG']	['Limb-girdle muscular dystrophy, type 2B']	246
NM_001040667.2(HSF4): c.256A > G (p.Ile86Val)	121909050	HSF4	['TTCGGAAGGTGGTG AGCRTCGAGCAGGG']	['Cataract, zonular']	247
NM_001363.4(DKC1): c.196A > G (p.Thr66Ala)	121912297	DKC1	['TTTGATAAGCTGAAT GTAAGGRCAACAC']	['Dyskeratosis congenita X-linked']	248
NM_001363.4(DKC1): c.361A > G (p.Ser121Gly)	121912305	DKC1	['TTCGGGTGGAGAAG ACAGGGCACRGTGG']	['Dyskeratosis congenita X-linked', 'Hoyeraal Hreidarsson syndrome']	249
NM_001943.3(DSG2): c.797A > G (p.Asn266Ser)	121913011	DSG2	['TTCGTATTTTGGATG TCARTGACAATAT']	['Arrhythmogenic right ventricular cardiomyopathy, type 10']	250
NM_000043.4(FAS): c.763A > G (p.Asn255Asp)	121913082	FAS	['TTTGTTCGAAAGAAT GGTGTCRATGAAG', 'TTCGAAAGAATGGTG TCRATGAAGCCAA']	[ ]	251, 252
NM_001127500.1(MET): c.3743A > G (p.Tyr1248Cys)	121913246	MET	['TTTGGTCTTGCCAGA GACATGTRTGATA']	['Renal cell carcinoma, papillary, 1']	253
NM_007035.3(KERA): c.740A > G (p.Asn247Ser)	121917858	KERA	['TTTGAGACTAAATCA CARCAAACTGTCA']	['Cornea plana 2']	254
NM_000536.3(RAG2): c.115A > G (p.Arg39Gly)	121917897	RAG2	['TTTGGACAAAAAGG CTGGCCCAAARGAT']	['Histiocytic medullary reticulosis']	255
NM_000040.1(APGC3): c.280A > G (p.Thr94Ala)	121918381	APOC3	['TTTGGACCCTGAGGT CAGACCARCTTCA']	[ ]	256
NM_021957.3(GYS2): c.116A > G (p.Asn39Ser)	121918423	GYS2	['TTTGAAGTTGCTTGG GAAGTGACCARTA']	['Hypoglycemia with deficiency of glycogen synthetase in the liver']	257
NM_002834.3(PTPN11): c.236A > G (p.Gln79Arg)	121918466	PTPN11	['TTTGCCACTTTGGCT GAGTTGGTCCRGT', 'TTTGGCTGAGTTGGT CCRGTATTACATG']	['Noonan syndrome', 'Noonan syndrome 1', 'Rasopathy', 'not provided']	258, 259
NM_000313.3(PROS1): c.586A > G (p.Lys196Glu)	121918474	PROS1	['TTTGTTATGCTTTCA AATRAGAAAGATT']	['Protein S deficiency']	260
NM_001063.3(TF): c.1936A > G (p.Lys646Glu)	121918678	TF	['TTTGTTTGTTCCGGT CGGAAACCRAGGA', TTTGTTCCGGTCGGA AACCRAGGACCTT']	[ ]	261, 262
NM_004612.3(TGFBR1): c.H99A > G (p.Asp400Gly)	121918711	TGFBR1	['TTTGAATCCTTCAAA CGTGCTGRCATCT']	['Loeys-Dietz syndrome 1']	263
NM_000532.4(PCCB): c.1304A > G (p.Tyr435Cys)	121964961	PCCB	['TTTGGATCTGTTTTA GGCCTRTGGAGGT']	['Propionic acidemia']	264

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_000137.2(FAH): c.836A > G (p.Gln279Arg)	121965078	FAH	['TTTGCTGTGCCCAAC CCGAAGCRGGTAA']	['Tyrosinemia type 1']	265
NM_000132.3(F8): c.1682A > G (p.Asp561Gly)	137852420	F8	['TTCGTTAATATGGAG AGAGRTCTAGCTT']	['Hereditary factor VIII deficiency disease']	266
NM_004387.3(NKX2-5):c.896A > G (p.Asp299Gly)	137852683	NKX2-5	['TTCGTGAACTTCGGC GTCGGGGRCTTGA']	['Atrial septal defect 7 with or without atrioventricular conduction defects']	267
NM_153638.2(PANK2): c.700A > G (p.Thr234Ala)	137852965	PANK2	['TTTGAACCCAAAGAC ATCRCTGCTGAAG']	[ ]	268
NM_001080463.1(DYNC2H1): c.11284A > G (p.Met3762Val)	137853026	DYNC2H1	['TTTGCTTTTGTAGGT TGCCRTGGGTCAA']	['Short-rib thoracic dysplasia 3 with or without polydactyly']	269
NM_031885.3(BBS2): c.472-2A > G	137854887	BBS2	['TTTGGGGTTTTATTT TCRGGTTACTGGA']	['Bardet-Biedl syndrome 2']	270
NM_000199.3(SGSH): c.892T > C (p.Ser298Pro)	138504221	SGSH	['TTTGGGTGCTCCGGG GRTGACACCAGTA']	['Mucopolysaccharidosis, MPS-III-A', 'not provided']	271
NM_004333.4(BRAF): c.770A > G (p.Gln257Arg)	180177035	BRAF	['TTTGTCGAAAGCTGC TTTTCCRGGGTTT']	['Noonan syndrome 7', 'Cardiofaciocutaneous syndrome', 'Rasopathy', 'not provided']	272
NM_003060.3(SLC22A5): c.694A > C (p.Thr232Pro)	188698686	SLC22A5	['TTCGTATAATATTCT CTRCGTTAGGAGT']	['not provided']	273
m.3243A > G	199474657	MT-TL1	['TTTGTTAAGATGGCA GRGCCCGGTAATC']	['Leigh disease', 'Cyclical vomiting syndrome', 'Juvenile myopathy, encephalopathy, lactic acidosis AND stroke', 'Myoclonus with epilepsy with ragged red fibers', 'Cytochrome-c oxidase deficiency', 'Diabetes-deafness syndrome maternally transmitted', '3-Methylglutaconic aciduria', 'Age-related macular degeneration 2', 'MERRF/MELAS overlap syndrome']	274
m.3252A > G	199474661	MT-TL1	['TTTGTTAAGATGGCA GAGCCCGGTARTC']	['Mitochondrial encephalomyopathy']	275
m.3251A > G	199474662	MT-TL1	['TTTGTTAAGATGGCA GAGCCCGGTRATC']	[ ]	276
NM_001018005.1(TPM1): c.742A > G (p.Lys248Glu)	199476319	TPM1	['TTTGCGGAGAGGTCA GTAACTRAATTGG']	['Left ventricular noncompaction 9', 'not provided']	277
NM_000267.3(NF1): c.1642-8A > G	267606602	NF1	['TTTGTCTTTCTCTTTT TTAAARAATTCA']; ['TTTGTCTTTCTCTTTT TTAAARAATTCA']	['Neurofibromatosis, type 1', 'Juvenile myelomonocytic leukemia']	278, 279
NM_198965.1(PTHLH): c.534A > G (p.Ter178Trp)	267606987	PTHLH	['TTTGCAGGAGGCATT GRAATTTTCAGCA']	['Brachydactyly type E2']	280

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_007373.3(SHOC2): c.4A > G (p.Ser2Gly)	267607048	SHOC2	['TTTGTCCAGGCTTGA GTCACCATGRGTA']	['Noonan-like syndrome with loose anagen hair', 'Rasopathy']	281
NM_018105.2(THAP1): c.266A > G (p.Lys89Arg)	267607111	THAP1	['TTTGTACTGAGCCAC ATGACARGGTAAT']	['Dystonia 6, torsion']	282
NM_003286.2(TOP1): c.1598A > G (p.Asp533Gly)	267607131	—	['TTTGACTTCCTCGGG AAGGRCTCCATCA']	[ ]	283
NM_000249.3(MLH1): c.545 + 3A > G	267607760	MLH1	['TTTGGAAGTTGTTGG CAGGTRCAGTCCA']	['Hereditary Nonpolyposis Colorectal Neoplasms']	284
NM_000288.3(PEX7): c.340-10A > G	267608255	PEX7	['TTTGCTTTCTAAACA CTTTTCARTGTTT']	['Phytanic acid storage disease', 'Peroxisome biogenesis disorder 9B']	285
NM_003159.2(CDKL5): c.464-2A > G	267608480	CDKL5	['TTTGGACTTTGCTAT CTTTCRGGTTTTG']	['Early infantile epileptic encephalopathy 2', 'not provided']	286
NM_212472.2(PRKAR1A: c.178-2A > G	281864796	PRKAR1A	['TTTGCAAACTCGTAA TTTCTTTCRGGAG']	['Carney complex, type 1']	287
NM_024312.4(GNPTAB): c.3458A > G (p.Asn1153Ser)	281865019	GNPTAB	['TTTGTTTGCCTGAAT GACARCATTGACC']	['Pseudo-Hurler polydystrophy']	288
NM_198578.3(LRRK2): c.5605A > G (p.Met1869Val)	281865052	LRRK2	['TTTGGCTGACCTGCC TAGAAATATTRTG']	['Parkinson disease 8, autosomal dominant']	289
NM_004614.4(TK2): c.562A > G (p.Thr188Ala)	281865495	TK2	['TTCGGACCAATCCTG AGRCTTGTTACCA']	['Mitochondrial DNA depletion syndrome 2']	290
NM_000495.4(COL4A5): c.610-2A > G	281874758	COL4A5	['TTTGTCTTCTCTTCTT RGGGCCCTCCTG']	['Alport syndrome, X-linked recessive']	291
NM_005359.5(SMAD4): c.1500A > G (p.Ile500Met)	281875320	SMAD4	['TTCGTCGCTTATGCA TRCTCAGGATGAG']	['Myhre syndrome', 'not provided']	292
NM_000095.2(CGMP): c.1760A > G (p.His587Arg)	312262901	COMP	['TTCGAGGGCACGTTC CRTGTGAACACGG']	['Pseudoachondroplastic spondyloepiphyseal dysplasia syndrome']	293
NM_000155.3(GALT): c.308A > G (p.Gln103Arg)	367543252	GALT	['TTTGACAACGACTTC CCAGCTCTGCRGC']	['Deficiency of UDPglucose-hexose-1-phosphate uridylyltransferase']	294
NM_018718.2(CEP41): c.107T > C (p.Met36Thr)	368178632	CEP41	['TTCGAGCTTCTCAGT ATATTTAGTCRTA']	['Joubert syndrome 9/15, digenic']	295
NM_005359.5(SMAD4): c.425-6A > G	377767327	SMAD4	['TTTGTTTTCCCCTTTA AACARTTAAGAT']	['Juvenile polyposis syndrome']	296
NM_002739.3(PRKCG): c.76A > G (p.Arg26Gly)	386134157	PRKCG	['TTTGCAGAAAGGGG GCCCTGRGGCAGAA']	['Spinocerebellar ataxia 14']	297
NM_000138.4(FBN1): c.5096A > G (p.Tyr1699Cys)	387906622	FBN1	['TTTGTGCTACAGAAA CTRCTATGCTGAC']	['Geleophysic dysplasia 2']	298
NM_000138.4(FBN1): c.5099A > G (p.Tyr1700Cys)	387906626	FBN1	['TTTGTGCTACAGAAA CTACTRTGCTGAC']	[ ]	299

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
m.10450A > G	387906731	MT-TR	['TTCGACTCATTAAAT TRTGATAATCATA']	['Mitochondrial encephalomyopathy']	300
m.5816A > G	387906732	MT-TC	['TTTGCAATTCAATAT GAAAATCRCCTCG']; ['TTTGCAATTCAATAT GAAAATCRCCTCG']	[ ]	301, 302
NM_001184.3(ATR): c.6431A > G (p.Gln2144Arg)	387906797	ATR	['TTTGACTGCTTTTTC ACRATTGATCTCT']	['Cutaneous telangiectasia and cancer syndrome, familial']	303
NM_006587.3(CORIN): c.1414A > G (p.Ser472Gly)	387906895	CORIN	['TTTGCCCTACAACAG TACARGTTATCCA']	['Preeclampsia/eclampsia 5']	304
NM_024700.3(SNIP1): c.1097A > G (p.Glu366Gly)	387906986	SNIP1	['TTTGGATTCAGTAGC AGAGRATACGTCT']	['Psychomotor retardation, epilepsy, and craniofacial dysmorphism']	305
NM_016952.4(CDON): c.2368A > G (p.Thr790Ala)	387906997	CDON	['TTTGTTTTCCCTCAA AGGTTCARCATAC']	['Holoprosencephaly 11']	306
NM_201269.2(ZNF644): c.2014A > G (p.Ser672Gly)	387907109	ZNF644	['TTTGGATCAACCTCA CAATCARGTAGTT']	['Myopia 21, autosomal dominant']	307
NM_016464.4(TMEM138): c.287A > G (p.His96Arg)	387907132	TMEM138	['TTTGCCCTCAGCATC TCCCTTCRTGTCT']	['Joubert syndrome 16']	308
NM_021629.3(GNB4):c. 265A > G (p.Lys89Glu)	387907341	GNB4	['TTTGGGATAGCTATA CAACAAATRAGGT']	['Charcot-Marie-Tooth disease, dominant intermediate F']	309
NM_032237.4(POMK): c.773A > G (p.Gln258Arg)	397509386	POMK	['TTCGTGGCTCCAGAG CRACTGTGGCCCT']	['Congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies, type A12']	310
NM_181690.2(AKT3): c.686A > G (p.Asn229Ser)	397514605	AKT3	['TTTGTGATGGAATAT GTTARTGGGGGCG']	['Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2']	311
NM_000138.4(FBN1): c.1148-2A > G	397515756	FBN1	['TTTGTTTTGTTGTGTT TTTCTRGAGGAT', 'TTTGTTGTGTTTTTCT RGAGGATTTCAA']	['Marfan syndrome']	312, 313
NM_000551.3(VHL): c.467A > G (p.Tyr156Cys)	397516441	VHL	['TTTGGTTTTTGCCCTT CCAGTGTRTACT', 'TTTGCCCTTCCAGTGT RTACTCTGAAAG']	['Von Hippel-Lindau syndrome']	314, 315
NM_033056.3(PCDH15): c.1998-2A > G	397517452	PCDH15	['TTTGTTTGTTTGTTTT GTCACTRGCACG', 'TTTGTTTGTTTTGTCA CTRGCACGGGGA']	['Usher syndrome, type IF']	316, 317
NM_000833.4(GRIN2A): c.1123-2A > G	397518469	GRIN2A	['TTTGTGTTTGTGCTG CRGGTGGGCAAGT']	['Focal epilepsy with speech disorder with or without mental retardation']	318

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_001204316.1 (PRLR): c.635A > G (p.His212Arg)	398122522	PRLR	['TTCGCTGCAAACCAG ACCRTGGATACTG']	['Hyperprolactinemia']	319
NM_000059.3(BRCA2): c.426-2A > G	398122779	BRCA2	['TTTGCTTTGTTTTATT TTRGTCCTGTTG']	['Familial cancer of breast', 'Breast-ovarian cancer, familial 2']	320
NM_000834.3(GRIN2B): c.2172-2A > G	398122824	GRIN2B	['TTTGTTTGCTTTTTTC CTGTACRGGAAA', 'TTTGCTTTTTTCCTGT ACRGGAAACTGG']	['Mental retardation, autosomal dominant 6']	321, 322
NM_003688.3(CASK): c.2168A > G (p.Tyr723Cys)	398122844	CASK	['TTTGATCAATTAGAT CTTGTCACATRTG']	['FG syndrome 4', 'Mental retardation and microcephaly with pontine and cerebellar hypoplasia']	323
NM_130466.3(UBE3B): c.545-2A > G	398123022	UBE3B	['TTTGTTCTCACTGTTT TCTTTCTTTRGG']	['Kaufman oculocerebrofacial syndrome']	324
NM_000350.2(ABCA4): c.67-2A > G	398123339	ABCA4	['TTTGTTTTGTTTTTCC RGATTCGCTTTG']	['Stargardt disease 1']	325
NC_000007.14: g.62535490A > G	483352872	—	['TTTGAGGCCTGTGGT TGARATGGAAATA']	['Isolated growth hormone deficiency type IB']	326
NM_001199397.1(NEK1): c.869-2A > G	483352906	NEK1	['TTTGTATCTTTTCATC TRGCTAAAAGAC']	['Short rib-polydactyly syndrome, Majewski type']	327
NM_015474.3(SAMHD1): c.1106T > C (p.Leu369Ser)	515726139	SAMHD1	['TTTGTGTTGATAAGC TCTACGGTGTRAA']	['Aicardi Goutieres syndrome 5']	328
NM_031229.2(RBCK1): c.1160A > G (p.Asn387Ser)	566912235	RBCK1	['TTTGAGGATGATGTC ARTGAGTTCACCT']	['Polyglucosan body myopathy 1 with or without immunodeficiency']	329
NM_001024847.2(TGFBR2): c.1472-2A > G	587776770	TGFBR2	['TTTGCTGGCTTTCTT CACRGAAGTAAAA']	['Loeys-Dietz syndrome 2']	330
NM_003094.3(SNRPE): c.1A > G (p.Met1Val)	587776924	SNRPE	['TTTGTGAAATTCCAC CRTGGCGTACCGT']	['Hypotrichosis 11']	331
NM_022068.3(PIEZO2): c.8215T > C (p.Ser2739Pro)	587777454	PIEZO2	['TTTGATCATTGTCTC TGGTGRGCGATAT']	['Oculomelic amyoplasia']	332
NM_152384.2(BBS5): c.522 + 3A > G	587777828	BBS5	['TTTGGAATTTATCCA GTGATCAGGTRTT']	['Bardet-Biedl syndrome 5']	333
NM_000136.2(FANCC): c.-78-2A > G	587779898	FANCC	['TTTGTTCCCTTTCTTA CRGATTTAATGT']	['Hereditary cancer-predisposing syndrome']	334
NM_058216.2(RAD51C): c.1027-2A > G	587780835	RAD51C	['TTTGTATATATATTT TTTATCTTTCRGC']	['Fanconi anemia, complementation group O']	335
NM_000060.3(BTD): c.683A > G (p.Asp228Gly)	587783004	BTD	['TTCGATGTTCCTCTT AAAGTGGRTCTCA']	['Biotinidase deficiency']	336

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.
SEQ ID NO(s):

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_003159.2(CDKL5): c.211A > G (p.Asn71Asp)	587783072	CDKL5	['TTCGGACTCTCAAGC AGGAARACATTGT']	['Atypical Rett syndrome', 'not provided']	337
NM_178151.2(DCX): c.1027-2A > G	587783518	DCX	['TTTGTCTCTTCTCTTC TTATRGGACCTG']	['Heterotopia']	338
NM_000252.2(MTM1): c.1406A > G (p.His469Arg)	587783789	MTM1	['TTTGATTATAATTTT GGATCRTCTGTAT']	['Severe X-linked myotubular myopathy']	339
NM_022458.3(LMBR1): c.423 + 5252A > G	606231150	LMBR1	['TTTGATCTTAGTGTT TATTACAGRAAAT']	['Triphalangeal thumb', 'Preaxial polydactyly 2']	340
NM_130466.3(UBE3B): c.1A > G (p.Met1Val)	672601304	UBE3B	['TTTGTGCAAGTTTGC AAACRTGTTCACC']	['Kaufman oculocerebrofacial syndrome']	341
NM_014305.3(TGDS): c.269A > G (p.Glu90Gly)	724160004	TGDS	['TTTGTGAAACTGCTT TTTGAAACAGRGA']	['Catel Manzke syndrome']	342
NM_014305.3(TGDS): c.892A > G (p.Asn298Asp)	724160005	TGDS	['TTTGTTTTCCAAGAC CCACCRATGACAT']	['Catel Manzke syndrome']	343
NM_001145901.1(SARS2): c.1175A > G (p.Asp392Gly)	727502784	SARS2	['TTCGTCCTTGACCGC AGGGTCCTGGRTA']	['Hyperuricemia, pulmonary hypertension, renal failure, and alkalosis']	344
NM_172362.2(KCNH1): c.1508A > G (p.Gln503Arg)	727502821	KCNH1	['TTCGGGAATGTGACG ACTATTTTCCRAC']; ['TTCGGGAATGTGACG ACTATTTTCCRAC']	['Temple-Baraitser syndrome']	345, 346
NM_000257.3(MYH7): c.1496A > G (p.Glu499Gly)	727504270	MYH7	['TTTGTGCTGGAGCAG GRGGAGTACAAGA']	['Cardiomyopathy', 'not specified']	347
NM_001110792.1 (MECP2): c.520A > G (p.Arg174Gly)	727505391	MECP2	['TTTGACTTCACGGTA ACTGGGRGAGGGA']	['Rett disorder']	348
NM_006343.2(MERTK): c.1605-2A > G	730880273	MERTK	['TTTGTTTTCATTCAC CCRGGAATGCATT']	['Retinitis pigmentosa 38']	349
NM_003611.2(OFD1): c.935 + 706A > G	730880283	OFD1	['TTTGGCAATAATGAA AAGGTAARTTGAT']	['Retinitis Pigmentosa 23']	350
NM_004793.3(LONP1): c.2353A > G (p.Arg785Gly)	730880293	LONP1	['TTTGTGGAGACATCC CTGRGACGGCCAC']	['CODAS syndrome']	351
NM_005633.3(SOS1): c.1430A > G (p.Gln477Arg)	730881044	SOSI	['TTTGCTGTAAATCAA ATCATGGGCRGCC']	['Rasopathy']	352
NM_012233.2(RAB3GAP1): c.649-2A > G	730882183	RAB3GA P1	['TTTGCATGTATTTCC TRGGGATGTCCTT']	['Warburg micro syndrome 1']	353
NM_016218.2(POLK): c.1679A > T (p.Glu560Val)	757103131	POLK	['TTTGTAAAACCTCTA GRAATGTCTCATA']	['Malignant tumor of prostate']	354
NM_003588.3(CUL4B): c.901-2A > G	786200913	CUL4B	['TTTGATTTCTTTTTTT TTCATTGGCRGA']	['Syndromic X-linked mental retardation, Cabezas type']	355

TABLE 2-continued

Human gene mutations that may be corrected by changing a guanineine (G) to a adenine (A) using GeoCas9 base editors provided herein. The gene name, gene symbol, and dbSNP database reference number (RS#) are indicated. Also indicated are exemplary protospacers with their PAM sequences (gRNAs) and the base to be edited, e.g., a G, indicated by a "R".The "gRNAs" sequences correspond to SEQ ID NOs: 200-373.

Name	RS# (dbSNP)	Gene Symbol	gRNA	Phenotypes	SEQ ID No(s):
NM_014946.3(SPAST): c.1165A > G (p.Thr389Ala)	786204132	SPAST	['TTTGGTCCACCTGGG AATGGGAAGRCAA']	['Spastic paraplegia 4, autosomal dominant']	356
NM_003816.2(ADAM9): c.411-8A > G	786205086	ADAM9	['TTTGTATACGTGTAA TGCARCATTCAGA']	[ ]	357
NM_003816.2(ADAM9): c.1396-2A > G	786205151	ADAM9	['TTTGAATTCTATTTC ACTRGTTCCTTCC']	[ ]	358
NM_003002.3(SDHD):c. 275A > G (p.Asp92Gly)	786205436	SDHD	['TTTGAATCCTTGCTC TGCGATGGRCTAT']	['Fatal infantile mitochondrial cardiomyopathy']	359
NM_016218.2(POLK): c.1477A > G (p.Lys493Glu)	786205692	POLK	['TTTGCCATTGCTAAG GAATTGCTARAAA']	['Malignant tumor of prostate']	360
NM_001604.5(PAX6): c.1075-2A > G	794726661	PAX6	['TTTGCCTCTCTCCTC ACRGCCCCCAGTC']	['Congenital aniridia']	361
NM_001165963.1(SCN1A): c.433A > G (p.Met145Val)	794726849	SCN1A	['TTTGACAAACTGTGT GTTTRTGACAATG']	['Severe myoclonic epilepsy in infancy']	362
NM_000138.4(FBN1): c.4337-2A > G	794728216	FBN1	['TTTGCTTTTTTCTCCC TCCCCCCARGAT']	['Thoracic aortic aneurysms and aortic dissections']	363
NM_000238.3(KCNH2): c.1900A > G (p.Thr634Ala)	794728377	KCNH2	['TTCGGCAACGTCTCT CCCAACRCCAACT']; ['TTCGGCAACGTCTCT CCCAACRCCAACT']	['Cardiac arrhythmia']	364, 365
NM_198056.2(SCN5A): c.4453A > G (p.Ile1485Val)	794728886	SCN5A	['TTTGCACTTAGGGGG CCAGGACRTCTTC']	['not provided']	366
NM_030973.3(MED25): c.116A > G (p.Tyr39Cys)	794729668	MED25	['TTCGAGGGGCTCCGC AAGCACTRCCTGC']	['BASEL-VANAGAITE-SMIRIN-YOSEF SYNDROME']	367
NM_006017.2(PROM1): c.2077-521A > G	796051882	PROM1	['TTTGCTGTTCTTCAC AGCTTTAGGRTAT']	['Cone-rod dystrophy 2']	368
NM_000016.5(ACADM): c.329A > G (p.Glu110Gly)	796051900	ACADM	['TTTGATGCTTGTTTA ATTAGTGRAGAAT']	['not provided']	369
NM_000833.4(GRIN2A): c.1930A > G (p.Ser644Gly)	796052544	GRIN2A	['TTCGCTGTCATATTC CTGGCTRGCTACA']	['not provided']	370
NM_021007.2(SCN2A): c.387-2A > G	796053169	SCN2A	['TTTGTCTTCCTTGAC GATATTCTRCTTT']; ['TTTGTCTTCCTTGAC GATATTCTRCTTT']	['not provided']	371, 372
NM_021007.2(SCN2A): c.851A > G (p.Asp284Gly)	796053173	SCN2A	['TTTGCAATGGCCTCC AGRTAATTCTTCC']	['not provided']	373

[0268] It will be apparent to those of skill in the art that in order to target a GeoCas9 base editor fusion protein as disclosed herein to a target site, e.g., a site comprising a point mutation to be edited, it is typically necessary to co-express the GeoCas9 fusion protein together with a guide RNA, e.g., an sgRNA. As explained in more detail elsewhere herein, a guide RNA typically comprises a tracrRNA framework allowing for Cas9 binding, and a guide sequence, which confers sequence specificity to the Cas9: nucleic acid editing enzyme/domain fusion protein. In some embodiments, the guide RNA comprises a structure 5'-[guide sequence]-[GUCAUAGUUCCCCUGAGAAAUCAGG-GUUACUAUGAUAAGGGCUUUCUGCCUA AGGCA-GACUGACCCGCGGCGUUGGGGAUCGCCUGU-

CGCCCGCUUUUGGGGGC
AUUCCCCAUCCUUUUUUU-3' (SEQ ID NO: 77)]-3', wherein the guide sequence comprises a sequence that is complementary to the target sequence. The guide sequence is typically 20 nucleotides long. The sequences of suitable guide RNAs for targeting GeoCas9: nucleic acid editing enzyme/domain fusion proteins to specific genomic target sites will be apparent to those of skill in the art based on the instant disclosure. Such suitable guide RNA sequences typically comprise guide sequences that are complementary to a nucleic sequence within 50 nucleotides upstream or downstream of the target nucleotide to be edited. Some exemplary guide RNA sequences suitable for targeting Cas9: nucleic acid editing enzyme/domain fusion proteins to specific target sequences are provided herein.

[0269] Some aspects of the disclosure provide methods for editing a nucleic acid. In some embodiments, the method is a method for editing a nucleobase of a nucleic acid (e.g., a base pair of a double-stranded DNA sequence). In some embodiments, the method comprises the steps of: a) contacting a target region of a nucleic acid (e.g., a double-stranded DNA sequence) with a complex comprising a base editor (e.g., a GeoCas9 domain fused to a cytidine deaminase domain) and a guide nucleic acid (e.g., gRNA), wherein the target region comprises a targeted nucleobase pair, b) inducing strand separation of said target region, c) converting a first nucleobase of said target nucleobase pair in a single strand of the target region to a second nucleobase, and d) cutting no more than one strand of said target region, where a third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase; and the method results in less than 20% indel formation in the nucleic acid. It should be appreciated that in some embodiments, step b is omitted. In some embodiments, the first nucleobase is a cytosine. In some embodiments, the second nucleobase is a deaminated cytosine, or a uracil. In some embodiments, the third nucleobase is a guanine. In some embodiments, the fourth nucleobase is an adenine. In some embodiments, the first nucleobase is a cytosine, the second nucleobase is a deaminated cytosine, or a uracil, the third nucleobase is a guanine, and the fourth nucleobase is an adenine. In some embodiments, the method results in less than 19%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, 4%, 2%, 1%, 0.5%, 0.2%, or less than 0.1% indel formation. In some embodiments, the method further comprises replacing the second nucleobase with a fifth nucleobase that is complementary to the fourth nucleobase, thereby generating an intended edited base pair (e.g., C: G->T: A). In some embodiments, the fifth nucleobase is a thymine. In some embodiments, at least 5% of the intended basepairs are edited. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the intended basepairs are edited.

[0270] In some embodiments, the ratio of intended products to unintended products in the target nucleotide is at least 2:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, 100:1, or 200:1, or more. In some embodiments, the ratio of intended point mutation to indel formation is greater than 1:1, 10:1, 50:1, 100:1, 500:1, or 1000:1, or more. In some embodiments, the cut single strand (nicked strand) is hybridized to the guide nucleic acid. In some embodiments, the cut single strand is opposite to the strand comprising the first nucleobase. In some embodiments, the base editor comprises a GeoCas9 domain. In some embodiments, the first base is cytosine, and the second base is not a G, C, A, or T. In some embodiments, the second base is uracil. In some embodiments, the first base is cytosine. In some embodiments, the second base is not a G, C, A, or T. In some embodiments, the second base is uracil. In some embodiments, the base editor inhibits base escision repair of the edited strand. In some embodiments, the base editor protects or binds the non-edited strand. In some embodiments, the base editor comprises UGI activity. In some embodiments, the base editor comprises nickase activity. In some embodiments, the intended edited basepair is upstream of a PAM site (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)). In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides upstream of the PAM site. In some embodiments, the intended edited basepair is downstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides downstream stream of the PAM site. In some embodiments, the method does not require a canonical GeoCas9 PAM site (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)). In some embodiments, the base editor comprises a linker. In some embodiments, the linker is 1-25 amino acids in length. In some embodiments, the linker is 5-20 amino acids in length. In some embodiments, linker is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In some embodiments, the target region comprises a target window, wherein the target window comprises the target nucleobase pair. In some embodiments, the target window comprises 1-10 nucleotides. In some embodiments, the target window is 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotides in length. In some embodiments, the target window is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the intended edited base pair is within the target window. In some embodiments, the target window comprises the intended edited base pair. In some embodiments, the method is performed using any of the base editors provided herein. In some embodiments, a target window is a deamination window

[0271] In some embodiments, the disclosure provides methods for editing a nucleotide. In some embodiments, the disclosure provides a method for editing a nucleobase pair of a double-stranded DNA sequence. In some embodiments, the method comprises a) contacting a target region of the double-stranded DNA sequence with a complex comprising a base editor and a guide nucleic acid (e.g., gRNA), where the target region comprises a target nucleobase pair, b) inducing strand separation of said target region, c) converting a first nucleobase of said target nucleobase pair in a single strand of the target region to a second nucleobase, d) cutting no more than one strand of said target region, wherein a third nucleobase complementary to the first nucleobase base is replaced by a fourth nucleobase complementary to the second nucleobase, and the second nucleobase is replaced with a fifth nucleobase that is complementary to the fourth nucleobase, thereby generating an intended edited basepair, wherein the efficiency of generating the intended edited basepair is at least 5%. It should be appreciated that in some embodiments, step b is omitted. In some embodiments, at least 5% of the intended basepaires are edited. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the intended basepaires are edited. In some embodiments, the method causes less than

19%, 18%, 16%, 14%, 12%, 10%, 8%, 6%, 4%, 2%, 1%, 0.5%, 0.2%, or less than 0.1% indel formation. In some embodiments, the ratio of intended product to unintended products at the target nucleotide is at least 2:1, 5:1, 10:1, 20:1, 30:1, 40:1, 50:1, 60:1, 70:1, 80:1, 90:1, 100:1, or 200:1, or more. In some embodiments, the ratio of intended point mutation to indel formation is greater than 1:1, 10:1, 50:1, 100:1, 500:1, or 1000:1, or more. In some embodiments, the cut single strand is hybridized to the guide nucleic acid. In some embodiments, the cut single strand is opposite to the strand comprising the first nucleobase. In some embodiments, the first base is cytosine. In some embodiments, the second nucleobase is not G, C, A, or T. In some embodiments, the second base is uracil. In some embodiments, the base editor inhibits base escision repair of the edited strand. In some embodiments, the base editor protects or binds the non-edited strand. In some embodiments, the nucleobase editor comprises UGI activity. In some embodiments, the nucleobase edit comprises nickase activity. In some embodiments, the intended edited basepair is upstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides upstream of the PAM site (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)). In some embodiments, the intended edited basepair is downstream of a PAM site. In some embodiments, the intended edited base pair is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides downstream stream of the PAM site. In some embodiments, the method does not require a canonical GeoCas9 PAM site (e.g., 5'-NNNNCRAA-3' (SEQ ID NO: 79)). In some embodiments, the nucleobase editor comprises a linker. In some embodiments, the linker is 1-25 amino acids in length. In some embodiments, the linker is 5-20 amino acids in length. In some embodiments, the linker is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In some embodiments, the target region comprises a target window, wherein the target window comprises the target nucleobase pair. In some embodiments, the target window comprises 1-10 nucleotides. In some embodiments, the target window is 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotides in length. In some embodiments, the target window is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length. In some embodiments, the intended edited base pair occurs within the target window. In some embodiments, the target window comprises the intended edited base pair. In some embodiments, the nucleobase editor is any one of the base editors provided herein.

V. Base Editor Efficiency

[0272] Some aspects of the disclosure are based on the recognition that any of the base editors provided herein are capable of modifying a specific nucleotide base without generating a significant proportion of indels. An "indel", as used herein, refers to the insertion or deletion of a nucleotide base within a nucleic acid. Such insertions or deletions can lead to frame shift mutations within a coding region of a gene. In some embodiments, it is desirable to generate base editors that efficiently modify (e.g. mutate or deaminate) a specific nucleotide within a nucleic acid, without generating a large number of insertions or deletions (i.e., indels) in the nucleic acid. In certain embodiments, any of the base editors provided herein are capable of generating a greater proportion of intended modifications (e.g., point mutations or deaminations) versus indels. In some embodiments, the base editors provided herein are capable of generating a ratio of intended point mutations to indels that is greater than 1:1. In some embodiments, the base editors provided herein are capable of generating a ratio of intended point mutations to indels that is at least 1.5:1, at least 2:1, at least 2.5:1, at least 3:1, at least 3.5:1, at least 4:1, at least 4.5:1, at least 5:1, at least 5.5:1, at least 6:1, at least 6.5:1, at least 7:1, at least 7.5:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1, at least 100:1, at least 200:1, at least 300:1, at least 400:1, at least 500:1, at least 600:1, at least 700:1, at least 800:1, at least 900:1, or at least 1000:1, or more. The number of intended mutations and indels may be determined using any suitable method known in the art

[0273] In some embodiments, the base editors provided herein are capable of limiting formation of indels in a region of a nucleic acid. In some embodiments, the region is at a nucleotide targeted by a base editor or a region within 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of a nucleotide targeted by a base editor. In some embodiments, any of the base editors provided herein are capable of limiting the formation of indels at a region of a nucleic acid to less than 1%, less than 1.5%, less than 2%, less than 2.5%, less than 3%, less than 3.5%, less than 4%, less than 4.5%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, less than 10%, less than 12%, less than 15%, or less than 20%. The number of indels formed at a nucleic acid region may depend on the amount of time a nucleic acid (e.g., a nucleic acid within the genome of a cell) is exposed to a base editor. In some embodiments, an number or proportion of indels is determined after at least 1 hour, at least 2 hours, at least 6 hours, at least 12 hours, at least 24 hours, at least 36 hours, at least 48 hours, at least 3 days, at least 4 days, at least 5 days, at least 7 days, at least 10 days, or at least 14 days of exposing a nucleic acid (e.g., a nucleic acid within the genome of a cell) to a base editor.

[0274] Some aspects of the disclosure are based on the recognition that any of the base editors provided herein are capable of efficiently generating an intended mutation, such as a point mutation, in a nucleic acid (e.g. a nucleic acid within a genome of a subject) without generating a significant number of unintended mutations, such as unintended point mutations. In some embodiments, a intended mutation is a mutation that is generated by a specific base editor bound to a gRNA, specifically designed to generate the intended mutation. In some embodiments, the intended mutation is a mutation associated with a disease or disorder. In some embodiments, the intended mutation is a cytosine (C) to thymine (T) point mutation associated with a disease or disorder. In some embodiments, the intended mutation is a guanine (G) to adenine (A) point mutation associated with a disease or disorder. In some embodiments, the intended mutation is a cytosine (C) to thymine (T) point mutation within the coding region of a gene. In some embodiments, the intended mutation is a guanine (G) to adenine (A) point mutation within the coding region of a gene. In some embodiments, the intended mutation is a point mutation that generates a stop codon, for example, a premature stop codon within the coding region of a gene. In some embodiments, the intended mutation is a mutation that eliminates a stop codon. In some embodiments, the intended mutation is a mutation that alters the splicing of a gene. In some embodiments, the intended mutation is a mutation that alters the

regulatory sequence of a gene (e.g., a gene promotor or gene repressor). In some embodiments, any of the base editors provided herein are capable of generating a ratio of intended mutations to unintended mutations (e.g., intended point mutations:unintended point mutations) that is greater than 1:1. In some embodiments, any of the base editors provided herein are capable of generating a ratio of intended mutations to unintended mutations (e.g., intended point mutations: unintended point mutations) that is at least 1.5:1, at least 2:1, at least 2.5:1, at least 3:1, at least 3.5:1, at least 4:1, at least 4.5:1, at least 5:1, at least 5.5:1, at least 6:1, at least 6.5:1, at least 7:1, at least 7.5:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1, at least 25:1, at least 30:1, at least 40:1, at least 50:1, at least 100:1, at least 150:1, at least 200:1, at least 250:1, at least 500:1, or at least 1000:1, or more. It should be appreciated that the characteristics of the base editors described in the "Base Editor Efficiency" section, herein, may be applied to any of the fusion proteins, or methods of using the fusion proteins provided herein.

VI. Pharmaceutical Compositions

[0275] Other aspects of the present disclosure relate to pharmaceutical compositions comprising any of the various components described herein (e.g., including, but not limited to, the GeoCas9 proteins, fusion proteins, guide RNAs, and complexes comprising fusion proteins and guide RNAs). In some embodiments, any of the fusion proteins, gRNAs, and/or complexes described herein are provided as part of a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprises any of the fusion proteins provided herein. In some embodiments, the pharmaceutical composition comprises any of the complexes provided herein. In some embodiments, the pharmaceutical composition comprises a ribonucleoprotein complex comprising an RNA-guided nuclease (e.g., GeoCas9) that forms a complex with a gRNA and a cationic lipid. In some embodiments pharmaceutical composition comprises a gRNA, a nucleic acid programmable DNA binding protein, a cationic lipid, and a pharmaceutically acceptable excipient. Pharmaceutical compositions may optionally comprise one or more additional therapeutically active substances.

[0276] As used here, the term "pharmaceutically-acceptable carrier" means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the compound from one site (e.g., the delivery site) of the body, to another site (e.g., organ, tissue or portion of the body). A pharmaceutically acceptable carrier is "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the tissue of the subject (e.g., physiologically compatible, sterile, physiologic pH, etc.). Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, methylcellulose, ethyl cellulose, microcrystalline cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium stearate, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol (PEG); (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates and/or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; (22) C2-C12 alcohols, such as ethanol; and (23) other non-toxic compatible substances employed in pharmaceutical formulations. Wetting agents, coloring agents, release agents, coating agents, sweetening agents, flavoring agents, perfuming agents, preservative and antioxidants can also be present in the formulation. The terms such as "excipient", "carrier", "pharmaceutically acceptable carrier" or the like are used interchangeably herein.

[0277] In some embodiments, compositions provided herein are administered to a subject, for example, to a human subject, in order to effect a targeted genomic modification within the subject. In some embodiments, cells are obtained from the subject and contacted with a any of the pharmaceutical compositions provided herein. In some embodiments, cells removed from a subject and contacted ex vivo with a pharmaceutical composition are re-introduced into the subject, optionally after the desired genomic modification has been effected or detected in the cells. Methods of delivering pharmaceutical compositions comprising nucleases are known, and are described, for example, in U.S. Pat. Nos. 6,453,242; 6,503,717; 6,534,261; 6,599,692; 6,607,882; 6,689,558; 6,824,978; 6,933,113; 6,979,539; 7,013,219; and 7,163,824, the disclosures of all of which are incorporated by reference herein in their entireties. Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally suitable for administration to animals or organisms of all sorts. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, domesticated animals, pets, and commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as chickens, ducks, geese, and/or turkeys.

[0278] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient(s) into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0279] Pharmaceutical formulations may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, includes any and all solvents, dispersion media,

diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's *The Science and Practice of Pharmacy,* 21st Edition, A. R. Gennaro (Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated in its entirety herein by reference) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. See also PCT application PCT/US2010/055131 (Publication number WO2011053982 A8, filed Nov. 2, 2010), incorporated in its entirety herein by reference, for additional suitable methods, reagents, excipients and solvents for producing pharmaceutical compositions comprising a nuclease. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this disclosure.

[0280] In some embodiments, compositions in accordance with the present invention may be used for treatment of any of a variety of diseases, disorders, and/or conditions, including but not limited to one or more of the following: autoimmune disorders (e.g. diabetes, lupus, multiple sclerosis, psoriasis, rheumatoid arthritis); inflammatory disorders (e.g. arthritis, pelvic inflammatory disease); infectious diseases (e.g. viral infections (e.g., HIV, HCV, RSV), bacterial infections, fungal infections, sepsis); neurological disorders (e.g. Alzheimer's disease, Huntington's disease; autism; Duchenne muscular dystrophy); cardiovascular disorders (e.g. atherosclerosis, hypercholesterolemia, thrombosis, clotting disorders, angiogenic disorders such as macular degeneration); proliferative disorders (e.g. cancer, benign neoplasms); respiratory disorders (e.g. chronic obstructive pulmonary disease); digestive disorders (e.g. inflammatory bowel disease, ulcers); musculoskeletal disorders (e.g. fibromyalgia, arthritis); endocrine, metabolic, and nutritional disorders (e.g. diabetes, osteoporosis); urological disorders (e.g. renal disease); psychological disorders (e.g. depression, schizophrenia); skin disorders (e.g. wounds, eczema); blood and lymphatic disorders (e.g. anemia, hemophilia); etc.

[0281] In other embodiments, the pharmaceutical composition described herein is delivered in a controlled release system. In one embodiment, a pump may be used (see, e.g., Langer, 1990, Science 249:1527-1533; Sefton, 1989, CRC Crit. Ref. Biomed. Eng. 14:201; Buchwald et al., 1980, Surgery 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used. (See, e.g., Medical Applications of Controlled Release (Langer and Wise eds., CRC Press, Boca Raton, Fla., 1974); Controlled Drug Bioavailability, Drug Product Design and Performance (Smolen and Ball eds., Wiley, New York, 1984); Ranger and Peppas, 1983, Macromol. Sci. Rev. Macromol. Chem. 23:61. See also Levy et al., 1985, Science 228:190; During et al., 1989, Ann. Neurol. 25:351; Howard et al., 1989, J. Neurosurg. 71:105.) Other controlled release systems are discussed, for example, in Langer, supra.

[0282] In some embodiments, the pharmaceutical composition is formulated in accordance with routine procedures as a composition adapted for intravenous or subcutaneous administration to a subject, e.g., a human. In some embodiments, pharmaceutical composition for administration by injection are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical can also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the pharmaceutical is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

[0283] A pharmaceutical composition for systemic administration may be a liquid, e.g., sterile saline, lactated Ringer's or Hank's solution. In addition, the pharmaceutical composition can be in solid forms and re-dissolved or suspended immediately prior to use. Lyophilized forms are also contemplated.

[0284] The pharmaceutical composition can be contained within a lipid particle or vesicle, such as a liposome or microcrystal, which is also suitable for parenteral administration. The particles can be of any suitable structure, such as unilamellar or plurilamellar, so long as compositions are contained therein. Compounds can be entrapped in "stabilized plasmid-lipid particles" (SPLP) containing the fusogenic lipid dioleoylphosphatidylethanolamine (DOPE), low levels (5-10 mol %) of cationic lipid, and stabilized by a polyethyleneglycol (PEG) coating (Zhang Y. P. et al., *Gene Ther.* 1999, 6:1438-47). Positively charged lipids such as N-[1-(2,3-dioleoyloxi) propyl]-N,N,N-trimethyl-amonium-methylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Pat. Nos. 4,880,635; 4,906,477; 4,911,928; 4,917,951; 4,920,016; and 4,921,757; each of which is incorporated herein by reference.

[0285] The pharmaceutical composition described herein may be administered or packaged as a unit dose, for example. The term "unit dose" when used in reference to a pharmaceutical composition of the present disclosure refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

[0286] Further, the pharmaceutical composition can be provided as a pharmaceutical kit comprising (a) a container containing a compound of the invention in lyophilized form and (b) a second container containing a pharmaceutically acceptable diluent (e.g., sterile water) for injection. The pharmaceutically acceptable diluent can be used for reconstitution or dilution of the lyophilized compound of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0287] In another aspect, an article of manufacture containing materials useful for the treatment of the diseases described above is included. In some embodiments, the article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials,

syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. In some embodiments, the container holds a composition that is effective for treating a disease described herein and may have a sterile access port. For example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle. The active agent in the composition is a compound of the invention. In some embodiments, the label on or associated with the container indicates that the composition is used for treating the disease of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution, or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

VII. Delivery Methods

[0288] In some aspects, the invention provides methods comprising delivering one or more polynucleotides, such as or one or more vectors as described herein encoding one or more components described herein, one or more transcripts thereof, and/or one or proteins transcribed therefrom, to a host cell. In some aspects, the invention further provides cells produced by such methods, and organisms (such as animals, plants, or fungi) comprising or produced from such cells. In some embodiments, a base editor as described herein in combination with (and optionally complexed with) a guide sequence is delivered to a cell. Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding components of a base editor to cells in culture, or in a host organism. Non-viral vector delivery systems include DNA plasmids, RNA (e.g. a transcript of a vector described herein), naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson, Science 256:808-813 (1992); Nabel & Felgner, TIBTECH 11:211-217 (1993); Mitani & Caskey, TIBTECH 11:162-166 (1993); Dillon, TIBTECH 11:167-175 (1993); Miller, Nature 357:455-460 (1992); Van Brunt, Biotechnology 6 (10): 1149-1154 (1988); Vigne, Restorative Neurology and Neuroscience 8:35-36 (1995); Kremer & Perricaudet, British Medical Bulletin 51 (1): 31-44 (1995); Haddada et al., in Current Topics in Microbiology and Immunology Doerfler and Bihm (eds) (1995); and Yu et al., Gene Therapy 1:13-26 (1994).

[0289] Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid: nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g. in vitro or ex vivo administration) or target tissues (e.g. in vivo administration). The preparation of lipid: nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[0290] The use of RNA or DNA viral based systems for the delivery of nucleic acids take advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (in vivo) or they can be used to treat cells in vitro, and the modified cells may optionally be administered to patients (ex vivo). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

[0291] The tropism of a viruses can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739 (1992); Johann et al., J. Virol. 66:1635-1640 (1992); Sommnerfelt et al., Virol. 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-2378 (1989); Miller et al., J. Virol. 65:2220-2224 (1991); PCT/US94/05700). In applications where transient expression is preferred, adenoviral based systems may be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors may also be used to transduce cells with target nucleic acids, e.g., in the in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy procedures (see, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et al., Mol. Cell. Biol. 4:2072-2081 (1984); Her-

monat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989).

[0292] Packaging cells are typically used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and w2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions are typically supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line may also be infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV. Additional methods for the delivery of nucleic acids to cells are known to those skilled in the art. See, for example, US20030087817, incorporated herein by reference.

VIII. Kits, Vectors, Cells

[0293] Some aspects of this disclosure provide kits comprising a nucleic acid construct, comprising (a) a nucleotide sequence encoding a GeoCas9 protein or a GeoCas9 fusion protein as provided herein; and (b) a heterologous promoter that drives expression of the sequence of (a). In some embodiments, the kit further comprises an expression construct encoding a guide RNA backbone, wherein the construct comprises a cloning site positioned to allow the cloning of a nucleic acid sequence identical or complementary to a target sequence into the guide RNA backbone.

[0294] Some aspects of this disclosure provide polynucleotides encoding a GeoCas9 protein of a fusion protein as provided herein. Some aspects of this disclosure provide vectors comprising such polynucleotides. In some embodiments, the vector comprises a heterologous promoter driving expression of polynucleotide.

[0295] Some aspects of this disclosure provide cells comprising a GeoCas9 protein, a fusion protein, a nucleic acid molecule encoding the fusion protein, a complex comprise the GeoCas9 protein and the gRNA, and/or a vector as provided herein.

[0296] The description of exemplary embodiments of the GeoCas9 system is provided for illustration purposes only and not meant to be limiting. Additional GeoCas9 systems, e.g., variations of the exemplary systems described in detail above, are also embraced by this disclosure. Some aspects of this disclosure provide kits comprising a nucleic acid construct comprising a nucleotide sequence encoding a GeoCas9 fusion protein editor described herein. In some embodiments, the nucleotide sequence comprises a heterologous promoter that drives expression of the GeoCas9 fusion proteins described herein.

[0297] Some aspects of this disclosure provide kits comprising a nucleic acid construct comprising a nucleotide sequence encoding a GeoCas9 fusion protein described herein. In some embodiments, the nucleotide sequence comprises a heterologous promoter that drives expression of the GeoCas9 fusion proteins.

[0298] Some aspects of this disclosure provide kits comprising a nucleic acid construct, comprising (a) a nucleotide sequence encoding a GeoCas9 (e.g., a GeoCas9 nickase) fused to a nucleic acid editing domain and (b) a heterologous promoter that drives expression of the sequence of (a). In some embodiments, the kit further comprises an expression construct encoding a guide RNA, or wherein the nucleic acid construct comprising the GeoCas9 fusion further comprises the nucleic acid sequence encoding the guide RNA. The promoters driving expression of the GeoCas9 fusion protein and the guide RNA can be the same of different.

[0299] Some aspects of this disclosure provide cells comprising any of the constructs disclosed herein. In some embodiments, a host cell is transiently or non-transiently transfected with one or more vectors described herein. In some embodiments, a cell is transfected as it naturally occurs in a subject. In some embodiments, a cell that is transfected is taken from a subject. In some embodiments, the cell is derived from cells taken from a subject, such as a cell line. A wide variety of cell lines for tissue culture are known in the art. Examples of cell lines include, but are not limited to, C8161, CCRF-CEM, MOLT, mIMCD-3, NHDF, HeLa-S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEKn, HEKa, MiaPaCell, Panc1, PC-3, TF1, CTLL-2, CIR, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu1, SW480, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, TIB55, Jurkat, J45.01, LRMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, 132-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, A2780ADR, A2780cis, A 172, A20, A253, A431, A-549, ALC, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293. BxPC3. C3H-10T1/2, C6/36, Cal-27, CHO, CHO-7, CHO—IR, CHO-K1, CHO-K2, CHO-T, CHO Dhfr−/−, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV-434, CML T1, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, HEK-293, HeLa, Hepalc1c7, HL-60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KYO1, LNCap, Ma-Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK 11, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NALM-1, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-5F, RMA/RMAS, Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-1, YAR, and transgenic varieties thereof. Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC) (Manassas, Va.)). In some embodiments, a cell transfected with one or more vectors described herein is used to establish a new cell line comprising one or more vector-derived sequences. In some embodiments, a cell transiently transfected with the components of a CRISPR

system as described herein (such as by transient transfection of one or more vectors, or transfection with RNA), and modified through the activity of a CRISPR complex, is used to establish a new cell line comprising cells containing the modification but lacking any other exogenous sequence. In some embodiments, cells transiently or non-transiently transfected with one or more vectors described herein, or cell lines derived from such cells are used in assessing one or more test compounds.

EQUIVALENTS AND SCOPE

[0300] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the embodiments described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the appended claims.

[0301] Articles such as "a," "an," and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between two or more members of a group are considered satisfied if one, more than one, or all of the group members are present, unless indicated to the contrary or otherwise evident from the context. The disclosure of a group that includes "or" between two or more group members provides embodiments in which exactly one member of the group is present, embodiments in which more than one members of the group are present, and embodiments in which all of the group members are present. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

[0302] It is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitation, element, clause, or descriptive term, from one or more of the claims or from one or more relevant portion of the description, is introduced into another claim. For example, a claim that is dependent on another claim can be modified to include one or more of the limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of making or using the composition according to any of the methods of making or using disclosed herein or according to methods known in the art, if any, are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[0303] Where elements are presented as lists, e.g., in Markush group format, it is to be understood that every possible subgroup of the elements is also disclosed, and that any element or subgroup of elements can be removed from the group. It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where an embodiment, product, or method is referred to as comprising particular elements, features, or steps, embodiments, products, or methods that consist, or consist essentially of, such elements, features, or steps, are provided as well. For purposes of brevity those embodiments have not been individually spelled out herein, but it will be understood that each of these embodiments is provided herein and may be specifically claimed or disclaimed.

[0304] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in some embodiments, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. For purposes of brevity, the values in each range have not been individually spelled out herein, but it will be understood that each of these values is provided herein and may be specifically claimed or disclaimed. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

[0305] In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Where ranges are given, any value within the range may explicitly be excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention, can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

EXAMPLES

Example 1—Construction of GeoCas9 Base Editors

Methods

[0306] Plasmids encoding GeoBE3 or GeoBE4, and a plasmid encoding a specific gRNA are transfected into HEK293T cells using Lipofectamine 2000 according to manufacturer's protocol. After 48 h, the cells were harvested and the genomic DNA was extracted. Target site amplicons were analyzed by Illumina high-throughput sequencing. Base editing efficiency was calculated using a custom Matlab program as used previously (Komor et al 2016).

Results

[0307] GeoBE3 and GeoBE4 can mediate efficient base editing on several genomic loci in HEK293T cells. GeoBE3 and GeoBE4 exhibits comparable base editing efficiencies. GeoBE3 and GeoBE4 has a wider window of deamination as evidenced by significant base editing occurring around the target site that is usually untargetable using SpCas9-based base editors. Base editing efficiencies varied from ~0 to 35%. See FIG. **3**.

[0308] GeoBE3 and GeoBE4 expands the targeting scope of base editing by providing access to additional PAM sites within the genome.

Exemplary Sequences

GeoCas9 single guide RNA (X denotes target sequence) 5' >3'
(SEQ ID NO: 435)
5'XXXXXXXXXXXXXXXXXXXXXXXXGTCATAGTTCCCCTGAGAAATCAG

GGTTACTATGATAAGGGCTTTCTGCCTAAGGCAGACTGACCCGCGGCGTTGGGGA

TCGCCTGTCGCCCGCTTTTGGCGGGCATTCCCCATCCTTTTTTT-3'
wherein X is A, U, C, or G.

GeoCas9 nickase (D8A)
(SEQ ID NO: 2)
RYKIGLAIGITSVGWAVMNLDIPRIEDLGVRIFDRAENPQTGESLALPRRLA

RSARRRLRRRKHRLERIRRLVIREGILTKEELDKLFEEKHEIDVWQLRVEALDRKLNN

DELARVLLHLAKRRGFKSNRKSERSNKENSTMLKHIEENRAILSSYRTVGEMIVKDP

KFALHKRNKGENYTNTIARDDLEREIRLIFSKQREFGNMSCTEEFENEYITIWASQRP

VASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIAWEHINKLRLISPSGARGLTDEER

RLLYEQAFQKNKITYHDIRTLLHLPDDTYFKGIVYDRGESRKQNENIRFLELDAYHQI

RKAVDKVYGKGKSSSFLPIDFDTFGYALTLFKDDADIHSYLRNEYEQNGKRMPNLA

NKVYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEVYSSACERAGYTFTGPKKK

QKTMLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARDLSQTFDERRKT

KKEQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQNGRCAYSLQPIEIERL

LEPGYVEVDHVIPYSRSLDDSYTNKVLVLTRENREKGNRIPAEYLGVGTERWQQFET

FVLTNKQFSKKKRDRLLRLHYDENEETEFKNRNLNDTRYISRFFANFIREHLKFAESD

DKQKVYTVNGRVTAHLRSRWEFNKNREESDLHHAVDAVIVACTTPSDIAKVTAFYQ

RREQNKELAKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGNYDDQKLESLQP

VFVSRMPKRSVTGAAHQETLRRYVGIDERSGKIQTVVKTKLSEIKLDASGHFPMYGK

ESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVKIIDTKNQVIPLND

GKTVAYNSNIVRVDVFEKDGKYYCVPVYTMDIMKGILPNKAIEPNKPYSEWKEMTE

DYTFRFSLYPNDLIRIELPREKTVKTAAGEEINVKDVFVYYKTIDSANGGLELISHDHR

FSLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVGLASSAHSKPGKTIRPLQSTRD

GeoBE3
(SEQ ID NO: 80)
MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWR

HTSQNTNKHVEVNFIEKFTTERYFCPNTRCSITWFLSWSPCGECSRAITEFLSRYPHVT

LFIYIARLYHHADPRNRQGLRDLISSGVTIQIMTEQESGYCWRNFVNYSPSNEAHWPR

YPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGL

KSGSETPGTSESATPESRYKIGLAIGITSVGWAVMNLDIPRIEDLGVRIFDRAENPQTG

ESLALPRRLARSARRRLRRRKHRLERIRRLVIREGILTKEELDKLFEEKHEIDVWQLR

VEALDRKLNNDELARVLLHLAKRRGFKSNRKSERSNKENSTMLKHIEENRAILSSYR

TVGEMIVKDPKFALHKRNKGENYTNTIARDDLEREIRLIFSKQREFGNMSCTEEFENE

YITIWASQRPVASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIA WEHINKLRLISPSG

ARGLTDEERRLLYEQAFQKNKITYHDIRTLLHLPDDTYFKGIVYDRGESRKQNENIRF

LELDAYHQIRKAVDKVYGKGKSSSFLPIDFDTFGYALTLFKDDADIHSYLRNEYEQN

GKRMPNLANKVYDNELIEELLNLSFTKFGHLSLKALRSILPYMEQGEVYSSACERAG

YTFTGPKKKQKTMLLPNIPPIANPVVMRALTQARKVVNAIIKKYGSPVSIHIELARDL

-continued

SQTFDERRKTKKEQDENRKKNETAIRQLMEYGLTLNPTGHDIVKFKLWSEQNGRCA

YSLQPIEIERLLEPGYVEVDHVIPYSRSLDDSYTNKVLVLTRENREKGNRIPAEYLGV

GTERWQQFETFVLTNKQFSKKKRDRLLRLHYDENEETEFKNRNLNDTRYISRFFANF

IREHLKFAESDDKQKVYTVNGRVTAHLRSRWEFNKNREESDLHHAVDAVIVACTTP

SDIAKVTAFYQRREQNKELAKKTEPHFPQPWPHFADELRARLSKHPKESIKALNLGN

YDDQKLESLQPVFVSRMPKRSVTGAAHQETLRRYVGIDERSGKIQTVVKTKLSEIKL

DASGHFPMYGKESDPRTYEAIRQRLLEHNNDPKKAFQEPLYKPKKNGEPGPVIRTVK

IIDTKNQVIPLNDGKTVAYNSNIVRVDVFEKDGKYYCVPVYTMDIMKGILPNKAIEPN

KPYSEWKEMTEDYTFRFSLYPNDLIRIELPREKTVKTAAGEEINVKDVFVYYKTIDSA

NGGLELISHDHRFSLRGVGSRTLKRFEKYQVDVLGNIYKVRGEKRVGLASSAHSKPG

KTIRPLQSTRDSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTA

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GeoBE4

(SEQ ID NO: 81)

MSSETGPVAVDPTLRRRIEPHEFEVFFDPRELRKETCLLYEINWGGRHSIWR

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YPHLWVRLYVLELYCIILGLPPCLNILRRKQPQLTFFTIALQSCHYQRLPPHILWATGL

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QREFGNMSCTEEFENEYITIWASQRPVASKDDIEKKVGFCTFEPKEKRAPKATYTFQS

FIAWEHINKLRLISPSGARGLTDEERRLLYEQAFQKNKITYHDIRTLLHLPDDTYFKGI

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DDADIHSYLRNEYEQNGKRMPNLANKVYDNELIEELLNLSFTKFGHLSLKALRSILPY

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KYGSPVSIHIELARDLSQTFDERRKTKKEQDENRKKNETAIRQLMEYGLTLNPTGHDI

VKFKLWSEQNGRCAYSLQPIEIERLLEPGYVEVDHVIPYSRSLDDSYTNKVLVLTREN

REKGNRIPAEYLGVGTERWQQFETFVLINKQFSKKKRDRLLRLHYDENEETEFKNR

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HHAVDAVIVACTTPSDIAKVTAFYQRREQNKELAKKTEPHFPQPWPHFADELRARLS

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EEVEEVIGNKPESDIL VHTAYDESTDENVMLLTSDAPEYKPWAL VIQDSNGENKIKM

LSGGSGGSGGSTNLSDIIEKETGKQLVIQESILMLPEEVEEVIGNKPESDILVHTAYDES

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SEQUENCE LISTING

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Sequence total quantity: 436
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source                  1..1368
                        mol_type = protein
                        organism = Streptococcus pyogenes
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ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG  120
NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD  180
VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN  240
LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI  300
LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA  360
GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH  420
AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE  480
VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL  540
SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI  600
IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG  660
RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL  720
HEHIANLAGS PAIKKGILQT VKVVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER  780
MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVDH  840
IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL  900
TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS  960
KLVSDFRKDF QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK  1020
MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF  1080
ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA  1140
YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK  1200
YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE  1260
QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA  1320
PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGD               1368

SEQ ID NO: 2            moltype = AA  length = 1086
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REGION                  1..1086
                        note = Synthetic
source                  1..1086
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 2
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RRKHRLERIR RLVIREGILT KEELDKLFEE KHEIDVWQLR VEALDRKLNN DELARVLLHL  120
AKRRGFKSNR KSERSNKENS TMLKHIEENR AILSSYRTVG EMIVKDPKFA LHKRNKGENY  180
TNTIARDDLE REIRLIFSKQ REFGNMSCTE EFENEYITIW ASQRPVASKD DIEKKVGFCT  240
FEPKEKRAPK ATYTFQSFIA WEHINKLRLI SPSGARGLTD EERRLLYEQA FQKNKITYHD  300
IRTLLHLPDD TYFKGIVYDR GESRKQNENI RFLELDAYHQ IRKAVDKVYG KGKSSSFLPI  360
DFDTFGYALT LFKDDADIHS YLRNEYEQNG KRMPNLANKV YDNELIEELL NLSFTKFGHL  420
SLKALRSILP YMEQGEVYSS ACERAGYTFT GPKKKQKTML LPNIPPIANP VVMRALTQAR  480
KVVNAIIKKY GSPVSIHIEL ARDLSQTFDE RRKTKKEQDE NRKKNETAIR QLMEYGLTLN  540
PTGHDIVKFK LWSEQNGRCA YSLQPIEIER LLEPGYVEVD HVIPYSRSLD DSYTNKVLVL  600
TRENREKGNR IPAEYLGVGT ERWQQFETFV LTNKQFSKKK RDRLLRLHYD ENEETEFKNR  660
NLNDTRYISR FFANFIREHL KFAESDDKQK VYTVNGRVTA HLRSRWEFNK NREESDLHHA  720
VDAVIVACTT PSDIAKVTAF YQRREQNKEL AKKTEPHFPQ PWPHFADELR ARLSKHPKES  780
IKALNLGNYD DQKLESLQPV FVSRMPKRSV TGAAHQETLR RYVGIDERSG KIQTVVKTKL  840
SEIKLDASGH FPMYGKESDP RTYEAIRQRL LEHNNDPKKA FQEPLYKPKK NGEPGPVIRT  900
VKIIDTKNQV IPLNDGKTVA YNSNIVRVDV FEKDGKYYCV PVYTMDIMKG ILPNKAIEPN  960
KPYSEWKEMT EDYTFRFSLY PNDLIRIELP REKTVKTAAG EEINVKDVFV YYKTIDSANG  1020
GLELISHDHR FSLRGVGSRT LKRFEKYQVD VLGNIYKVRG EKRVGLASSA HSKPGKTIRP  1080
LQSTRD                                                             1086
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SEQ ID NO: 3            moltype = AA  length = 1086
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                        note = Synthetic
source                  1..1086
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                        organism = synthetic construct
SEQUENCE: 3
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RRKHRLERIR RLVIREGILT KEELDKLFEE KHEIDVWQLR VEALDRKLNN DELARVLLHL  120
AKRRGFKSNR KSERSNKENS TMLKHIEENR AILSSYRTVG EMIVKDPKFA LHKRNKGENY  180
TNTIARDDLE REIRLIFSKQ REFGNMSCTE EFENEYITIW ASQRPVASKD DIEKKVGFCT  240
FEPKEKRAPK ATYTFQSFIA WEHINKLRLI SPSGARGLTD EERRLLYEQA FQKNKITYHD  300
IRTLLHLPDD TYFKGIVYDR GESRKQNENI RFLELDAYHQ IRKAVDKVYG KGKSSSFLPI  360
DFDTFGYALT LFKDDADIHS YLRNEYEQNG KRMPNLANKV YDNELIEELL NLSFTKFGHL  420
SLKALRSILP YMEQGEVYSS ACERAGYTFT GPKKKQKTML LPNIPPIANP VVMRALTQAR  480
KVVNAIIKKY GSPVSIHIEL ARDLSQTFDE RRKTKKEQDE NRKKNETAIR QLMEYGLTLN  540
PTGHDIVKFK LWSEQNGRCA YSLQPIEIER LLEPGYVEVD HVIPYSRSLD DSYTNKVLVL  600
TRENREKGNR IPAEYLGVGT ERWQQFETFV LTNKQFSKKK RDRLLRLHYD ENEETEFKNR  660
NLNDTRYISR FFANFIREHL KFAESDDKQK VYTVNGRVTA HLRSRWEFNK NREESDLHHA  720
VDAVIVACTT PSDIAKVTAF YQRREQNKEL AKKTEPHFPQ PWPHFADELR ARLSKHPKES  780
IKALNLGNYD DQKLESLQPV FVSRMPKRSV TGAAHQETLR RYVGIDERSG KIQTVVKTKL  840
SEIKLDASGH FPMYGKESDP RTYEAIRQRL LEHNNDPKKA FQEPLYKPKK NGEPGPVIRT  900
VKIIDTKNQV IPLNDGKTVA YNSNIVRVDV FEKDGKYYCV PVYTMDIMKG ILPNKAIEPN  960
KPYSEWKEMT EDYTFRFSLY PNDLIRIELP REKTVKTAAG EEINVKDVFV YYKTIDSANG  1020
GLELISHDHR FSLRGVGSRT LKRFEKYQVD VLGNIYKVRG EKRVGLASSA HSKPGKTIRP  1080
LQSTRD                                                             1086

SEQ ID NO: 4            moltype = DNA  length = 3249
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source                  1..3249
                        mol_type = genomic DNA
                        note = Bacillus thermodenitrificans
                        organism = unidentified
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aaaaccgggg agtcactagc tcttccacgt cgcctcgccc gctccgcccg acgtcgtctg  180
cggcgtcgca aacatcgact ggagcgcatt cgccgcctgt tcgtccgcga aggaatttta  240
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tacaccaaca ctgttgcccg cgacgatctt gaacgggaaa tcaaactgat tttcgccaaa  600
cagcgcgaat atgggaacat cgtttgcaca gaagcatttg aacacgagta tatttccatt  660
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gtctgggaac atattaacaa acttcgtctt gtctccccgg gaggcatccg ggcactaacc  840
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gcccatttac gcagccgttg gaattttaac aaaaaccggg aagaatcgaa tttgcatcat  2160
gccgtcgatg ctgccatcgt cgcctgcaca acgccgagcg atatcgcccg agtcaccgcc  2220
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SEQ ID NO: 5            moltype = AA  length = 1082
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source                  1..1082
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                        note = Bacillus thermodenitrificans
                        organism = unidentified
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LAKRRGFRSN RKSERTNKEN STMLKHIEEN QSILSSYRTV AEMVVKDPKF SLHKRNKEDN  180
YTNTVARDDL EREIKLIFAK QREYGNIVCT EAFEHEYISI WASQRPFASK DDIEKKVGFC  240
TFEPKEKRAP KATYTFQSFT VWEHINKLRL VSPGGIRALT DDERRLIYKQ AFHKNKITFH  300
DVRTLLNLPD DTRFKGLLYD RNTTLKENEK VRFLELGAYH KIRKAIDSVY GKGAAKSFRP  360
IDFDTFGYAL TMFKDDTDIR SYLRNEYEQN GKRMENLADK VYDEELIEEL LNLSFSKFGH  420
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RKVVNAIIKK YGSPVSIHIE LARELSQSFD ERRKMQKEQE GNRKKNETAI RQLVEYGLTL  540
NPTGLDIVKF KLWSEQNGKC AYSLQPIEIE RLLEPGYTEV DHVIPYSRSL DDSYTNKVLV  600
LTKENREKGN RTPAEYLGLG SERWQQFETF VLTNKQFSKK KRDRLLRLHY DENEENEFKN  660
RNLNDTRYIS RFLANFIREH LKFADSDDKQ KVYTVNGRIT AHLRSRWNFN KNREESNLHH  720
AVDAAIVACT TPSDIARVTA FYQRREQNKE LSKKTDPQFP QPWPHFADEL QARLSKNPKE  780
SIKALNLGNY DNEKLESLQP VFVSRMPKRS ITGAAHQETL RRYIGIDERS GKIQTVVKKK  840
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TIKIIDTTNQ VIPLNDGKTV AYNSNIVRVD VFEKDGKYYC VPIYTIDMMK GILPNKAIEP  960
NKPYSEWKEM TEDYTFRFSL YPNDLIRIEF PREKTIKTAV GEEIKIKDLF AYYQTIDSSN  1020
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SEQ ID NO: 6            moltype = AA  length = 1087
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source                  1..1087
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                        organism = unidentified
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LAKRRGFKSN RKSERSNKEN STMLKHIEEN RAILSSYRTV GEMIVKDPKF ALHKRNKGEN  180
YTNTIARDDL EREIRLIFSK QREFGNMSCT EEFENEYITI WASQRPVASK DDIEKKVGFC  240
TFEPKEKRAP KATYTFQSFI AWEHINKLRL ISPSGARGLT DEERRLLYEQ AFQKNKITYH  300
DIRTLLHLPD DTYFKGIVYD RGESRKQNEN IRFLELDAYH QIRKAVDKVY GKGKSSSFLP  360
IDFDTFGYAL TLFKDDADIH SYLRNEYEQN GKRMPNLANK VYDNELIEEL LNLSFTKFGH  420
LSLKALRSIL PYMEQGEVYS SACERAGYTF TGPKKKQKTM LLPNIPPIAN PVVMRALTQA  480
RKVVNAIIKK YGSPVSIHIE LARDLSQTFD ERRKTKKEQD ENRKKNETAI RQLMEYGLTL  540
NPTGHDIVKF KLWSEQNGRC AYSLQPIEIE RLLEPGYVEV DHVIPYSRSL DDSYTNKVLV  600
LTRENREKGN RIPAEYLGVG TERWQQFETF VLTNKQFSKK KRDRLLRLHY DENEETEFKN  660
RNLNDTRYIS RFFANFIREH LKFAESDDKQ KVYTVNGRVT AHLRSRWEFN KNREESDLHH  720
AVDAAIVACT TPSDIAKVTA FYQRREQNKE LAKKTEPHFP QPWPHFADEL RARLSKHPKE  780
SIKALNLGNY DDQKLESLQP VFVSRMPKRS VTGAAHQETL RRYVGIDERS GKIQTVVKTK  840
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NKPYSEWKEM TEDYTFRFSL YPNDLIRIEL PREKTVKTAA GEEINVKDVF VYYKTIDSAN  1020
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SEQ ID NO: 7            moltype = AA  length = 1082
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                        organism = synthetic construct
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YTNTVARDDL EREIKLIFAK QREYGNIVCT EAFEHEYISI WASQRPFASK DDIEKKVGFC  240
TFEPKEKRAP KATYTFQSFT VWEHINKLRL VSPGGIRALT DDERRLIYKQ AFHKNKITFH  300
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IDFDTFGYAL TMFKDDTDIR SYLRNEYEQN GKRMENLADK VYDEELIEEL LNLSFSKFGH  420
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RKVVNAIIKK YGSPVSIHIE LARELSQSFD ERRKMQKEQE GNRKKNETAI RQLVEYGLTL  540
NPTGLDIVKF KLWSEQNGKC AYSLQPIEIE RLLEPGYTEV DAVIPYSRSL DDSYTNKVLV  600
LTKENREKGN RTPAEYLGLG SERWQQFETF VLTNKQFSKK KRDRLLRLHY DENEENEFKN  660
RNLNDTRYIS RFLANFIREH LKFADSDDKQ KVYTVNGRIT AHLRSRWNFN KNREESNLHH  720
AVDAAIVACT TPSDIARVTA FYQRREQNKE LSKKTDPQFP QPWPHFADEL QARLSKNPKE  780
SIKALNLGNY DNEKLESLQP VFVSRMPKRS ITGAAHQETL RRYIGIDERS GKIQTVVKKK  840
LSEIQLDKTG HFPMYGKESD PRTYEAIRQR LLEHNNDPKK AFQEPLYKPK KNGELGPIIR  900
TIKIIDTTNQ VIPLNDGKTV AYNSNIVRVD VFEKDGKYYC VPIYTIDMMK GILPNKAIEP  960
NKPYSEWKEM TEDYTFRFSL YPNDLIRIEF PREKTIKTAV GEEIKIKDLF AYYQTIDSSN  1020
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PL                                                                 1082

SEQ ID NO: 8            moltype = AA  length = 1082
FEATURE                 Location/Qualifiers
REGION                  1..1082
                        note = Synthetic
source                  1..1082
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 8
MKYKIGLAIG ITSIGWAVIN LDIPRIEDLG VRIFDRAENP KTGESLALPR RLARSARRRL  60
RRRKHRLERI RRLFVREGIL TKEELNKLFE KKHEIDVWQL RVEALDRKLN NDELARILLH  120
LAKRRGFRSN RKSERTNKEN STMLKHIEEN QSILSSYRTV AEMVVKDPKF SLHKRNKEDN  180
YTNTVARDDL EREIKLIFAK QREYGNIVCT EAFEHEYISI WASQRPFASK DDIEKKVGFC  240
TFEPKEKRAP KATYTFQSFT VWEHINKLRL VSPGGIRALT DDERRLIYKQ AFHKNKITFH  300
DVRTLLNLPD DTRFKGLLYD RNTTLKENEK VRFLELGAYH KIRKAIDSVY GKGAAKSFRP  360
IDFDTFGYAL TMFKDDTDIR SYLRNEYEQN GKRMENLADK VYDEELIEEL LNLSFSKFGH  420
LSLKALRNIL PYMEQGEVYS TACERAGYTF TGPKKKQKTV LLPNIPPIAN PVVMRALTQA  480
RKVVNAIIKK YGSPVSIHIE LARELSQSFD ERRKMQKEQE GNRKKNETAI RQLVEYGLTL  540
NPTGLDIVKF KLWSEQNGKC AYSLQPIEIE RLLEPGYTEV DHVIPYSRSL DDSYTNKVLV  600
LTKENREKGN RTPAEYLGLG SERWQQFETF VLTNKQFSKK KRDRLLRLHY DENEENEFKN  660
RNLNDTRYIS RFLANFIREH LKFADSDDKQ KVYTVNGRIT AHLRSRWNFN KNREESNLHH  720
AVDAAIVACT TPSDIARVTA FYQRREQNKE LSKKTDPQFP QPWPHFADEL QARLSKNPKE  780
SIKALNLGNY DNEKLESLQP VFVSRMPKRS ITGAAHQETL RRYIGIDERS GKIQTVVKKK  840
LSEIQLDKTG HFPMYGKESD PRTYEAIRQR LLEHNNDPKK AFQEPLYKPK KNGELGPIIR  900
TIKIIDTTNQ VIPLNDGKTV AYNSNIVRVD VFEKDGKYYC VPIYTIDMMK GILPNKAIEP  960
NKPYSEWKEM TEDYTFRFSL YPNDLIRIEF PREKTIKTAV GEEIKIKDLF AYYQTIDSSN  1020
GGLSLVSHDN NFSLRSIGSR TLKRFEKYQV DVLGNIYKVR GEKRVGVASS SHSKAGETIR  1080
PL                                                                 1082

SEQ ID NO: 9            moltype = AA  length = 124
FEATURE                 Location/Qualifiers
REGION                  1..124
                        note = Synthetic
REGION                  5..124
                        note = may or may not be present
source                  1..124
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 9
GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS  60
GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS GGGSGGGSGG GSGGGSGGGS  120
GGGS                                                               124

SEQ ID NO: 10           moltype = AA  length = 155
FEATURE                 Location/Qualifiers
REGION                  1..155
                        note = Synthetic
REGION                  6..155
                        note = may or may not be present
source                  1..155
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 10
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS  60
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS  120
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGS                             155
```

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SEQ ID NO: 11           moltype = AA  length = 31
FEATURE                 Location/Qualifiers
REGION                  1..31
                        note = Synthetic
REGION                  2..31
                        note = may or may not be present
source                  1..31
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 11
GGGGGGGGGG GGGGGGGGGG GGGGGGGGGG G                                  31

SEQ ID NO: 12           moltype = AA  length = 155
FEATURE                 Location/Qualifiers
REGION                  1..155
                        note = Synthetic
REGION                  6..155
                        note = may or may not be present
source                  1..155
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 12
EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK  60
EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK  120
EAAAKEAAAK EAAAKEAAAK EAAAKEAAAK EAAAK                             155

SEQ ID NO: 13           moltype = AA  length = 93
FEATURE                 Location/Qualifiers
REGION                  1..93
                        note = Synthetic
REGION                  4..93
                        note = may or may not be present
source                  1..93
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 13
GGSGGSGGSG GSGGSGGSGG SGGSGGSGGS GGSGGSGGSG GSGGSGGSGG SGGSGGSGGS  60
GGSGGSGGSG GSGGSGGSGG SGGSGGSGGS GGS                               93

SEQ ID NO: 14           moltype = AA  length = 124
FEATURE                 Location/Qualifiers
REGION                  1..124
                        note = Synthetic
REGION                  5..124
                        note = may or may not be present
source                  1..124
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 14
SGGSSGGSSG GSSGGSSGGS SGGSSGGSSG GSSGGSSGGS SGGSSGGSSG GSSGGSSGGS  60
SGGSSGGSSG GSSGGSSGGS SGGSSGGSSG GSSGGSSGGS SGGSSGGSSG GSSGGSSGGS  120
SGGS                                                               124

SEQ ID NO: 15           moltype = AA  length = 16
FEATURE                 Location/Qualifiers
REGION                  1..16
                        note = Synthetic
source                  1..16
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 15
SGSETPGTSE SATPES                                                  16

SEQ ID NO: 16           moltype = AA  length = 97
FEATURE                 Location/Qualifiers
REGION                  1..97
                        note = Synthetic
REGION                  8..97
                        note = may or may not be present
source                  1..97
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 16
SGGSGGSGGS GGSGGSGGSG GSGGSGGSGG SGGSGGSGGS GGSGGSGGSG GSGGSGGSGG  60
SGGSGGSGGS GGSGGSGGSG GSGGSGGSGG SGGSGGS                           97
```

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```
SEQ ID NO: 17          moltype = AA  length = 32
FEATURE                Location/Qualifiers
REGION                 1..32
                       note = Synthetic
source                 1..32
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 17
SGGSSGGSSG SETPGTSESA TPESSGGSSG GS                                32

SEQ ID NO: 18          moltype = AA  length = 62
FEATURE                Location/Qualifiers
REGION                 1..62
                       note = Synthetic
VARIANT                1
                       note = X can be any naturally occurring amino acid
REGION                 3..62
                       note = may or may not be present
VARIANT                3
                       note = X can be any naturally occurring amino acid
VARIANT                5
                       note = X can be any naturally occurring amino acid
VARIANT                7
                       note = X can be any naturally occurring amino acid
VARIANT                9
                       note = X can be any naturally occurring amino acid
VARIANT                11
                       note = X can be any naturally occurring amino acid
VARIANT                13
                       note = X can be any naturally occurring amino acid
VARIANT                15
                       note = X can be any naturally occurring amino acid
VARIANT                17
                       note = X can be any naturally occurring amino acid
VARIANT                19
                       note = X can be any naturally occurring amino acid
VARIANT                21
                       note = X can be any naturally occurring amino acid
VARIANT                23
                       note = X can be any naturally occurring amino acid
VARIANT                25
                       note = X can be any naturally occurring amino acid
VARIANT                27
                       note = X can be any naturally occurring amino acid
VARIANT                29
                       note = X can be any naturally occurring amino acid
VARIANT                31
                       note = X can be any naturally occurring amino acid
VARIANT                33
                       note = X can be any naturally occurring amino acid
VARIANT                35
                       note = X can be any naturally occurring amino acid
VARIANT                37
                       note = X can be any naturally occurring amino acid
VARIANT                39
                       note = X can be any naturally occurring amino acid
VARIANT                41
                       note = X can be any naturally occurring amino acid
VARIANT                43
                       note = X can be any naturally occurring amino acid
VARIANT                45
                       note = X can be any naturally occurring amino acid
VARIANT                47
                       note = X can be any naturally occurring amino acid
VARIANT                49
                       note = X can be any naturally occurring amino acid
VARIANT                51
                       note = X can be any naturally occurring amino acid
VARIANT                53
                       note = X can be any naturally occurring amino acid
VARIANT                55
                       note = X can be any naturally occurring amino acid
VARIANT                57
                       note = X can be any naturally occurring amino acid
VARIANT                59
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                        note = X can be any naturally occurring amino acid
VARIANT                 61
                        note = X can be any naturally occurring amino acid
source                  1..62
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 18
XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP XPXPXPXPXP  60
XP                                                                 62

SEQ ID NO: 19           moltype = AA  length = 24
FEATURE                 Location/Qualifiers
REGION                  1..24
                        note = Synthetic
REGION                  4..24
                        note = may or may not be present
source                  1..24
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 19
GGSGGSGGSG GSGGSGGSGG SGGS                                         24

SEQ ID NO: 20           moltype = AA  length = 48
FEATURE                 Location/Qualifiers
REGION                  1..48
                        note = Synthetic
REGION                  4..48
                        note = may or may not be present
source                  1..48
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 20
GGSGGSGGSG GSGGSGGSGG SGGSGGSGGS GGSGGSGGSG GSGGSGGS               48

SEQ ID NO: 21           moltype = AA  length = 34
FEATURE                 Location/Qualifiers
REGION                  1..34
                        note = Synthetic
REGION                  8..34
                        note = may or may not be present
source                  1..34
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 21
SGGSGGSGGS GGSGGSGGSG GSGGSGGSGG SGGS                              34

SEQ ID NO: 22           moltype = AA  length = 13
FEATURE                 Location/Qualifiers
REGION                  1..13
                        note = Synthetic
REGION                  8..13
                        note = may or may not be present
source                  1..13
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 22
SGGSGGSGGS GGS                                                     13

SEQ ID NO: 23           moltype = AA  length = 11
FEATURE                 Location/Qualifiers
REGION                  1..11
                        note = Synthetic
source                  1..11
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 23
SPKKKRKVEA S                                                       11

SEQ ID NO: 24           moltype = AA  length = 10
FEATURE                 Location/Qualifiers
REGION                  1..10
                        note = Synthetic
REGION                  8..10
                        note = may or may not be present
source                  1..10
                        mol_type = protein
```

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```
                        organism = synthetic construct
SEQUENCE: 24
SGGSGGSGGS                                                          10

SEQ ID NO: 25           moltype = AA  length = 198
FEATURE                 Location/Qualifiers
source                  1..198
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 25
MDSLLMNRRK FLYQFKNVRW AKGRRETYLC YVVKRRDSAT SFSLDFGYLR NKNGCHVELL  60
FLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVADFLRG NPNLSLRIFT ARLYFCEDRK  120
AEPEGLRRLH RAGVQIAIMT FKDYFYCWNT FVENHERTFK AWEGLHENSV RLSRQLRRIL  180
LPLYEVDDLR DAFRTLGL                                                198

SEQ ID NO: 26           moltype = AA  length = 198
FEATURE                 Location/Qualifiers
source                  1..198
                        mol_type = protein
                        organism = Mus musculus
SEQUENCE: 26
MDSLLMKQKK FLYHFKNVRW AKGRHETYLC YVVKRRDSAT SCSLDFGHLR NKSGCHVELL  60
FLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVAEFLRW NPNLSLRIFT ARLYFCEDRK  120
AEPEGLRRLH RAGVQIGIMT FKDYFYCWNT FVENRERTFK AWEGLHENSV RLTRQLRRIL  180
LPLYEVDDLR DAFRMLGF                                                198

SEQ ID NO: 27           moltype = AA  length = 198
FEATURE                 Location/Qualifiers
source                  1..198
                        mol_type = protein
                        organism = Canis lupus
SEQUENCE: 27
MDSLLMKQRK FLYHFKNVRW AKGRHETYLC YVVKRRDSAT SFSLDFGHLR NKSGCHVELL  60
FLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVADFLRG YPNLSLRIFA ARLYFCEDRK  120
AEPEGLRRLH RAGVQIAIMT FKDYFYCWNT FVENREKTFK AWEGLHENSV RLSRQLRRIL  180
LPLYEVDDLR DAFRTLGL                                                198

SEQ ID NO: 28           moltype = AA  length = 199
FEATURE                 Location/Qualifiers
source                  1..199
                        mol_type = protein
                        organism = Bos taurus
SEQUENCE: 28
MDSLLKKQRQ FLYQFKNVRW AKGRHETYLC YVVKRRDSPT SFSLDFGHLR NKAGCHVELL  60
FLRYISDWDL DPGRCYRVTW FTSWSPCYDC ARHVADFLRG YPNLSLRIFT ARLYFCDKER  120
KAEPEGLRRL HRAGVQIAIM TFKDYFYCWN TFVENHERTF KAWEGLHENS VRLSRQLRRI  180
LLPLYEVDDL RDAFRTLGL                                               199

SEQ ID NO: 29           moltype = AA  length = 429
FEATURE                 Location/Qualifiers
source                  1..429
                        mol_type = protein
                        organism = Mus musculus
SEQUENCE: 29
MGPFCLGCSH RKCYSPIRNL ISQETFKFHF KNLGYAKGRK DTFLCYEVTR KDCDSPVSLH  60
HGVFKNKDNI HAEICFLYWF HDKVLKVLSP REEFKITWYM SWSPCFECAE QIVRFLATHH  120
NLSLDIFSSR LYNVQDPETQ QNLCRLVQEG AQVAAMDLYE FKKCWKKFVD NGGRRFRPWK  180
RLLTNFRYQD SKLQEILRPC YIPVPSSSSS TLSNICLTKG LPETRFCVEG RRMDPLSEEE  240
FYSQFYNQRV KHLCYYHRMK PYLCYQLEQF NGQAPLKGCL LSEKGKQHAE ILFLDKIRSM  300
ELSQVTITCY LTWSPCPNCA WQLAAFKRDR PDLILHIYTS RLYFHWKRPF QKGLCSLWQS  360
GILVDVMDLP QFTDCWTNFV NPKRPFWPWK GLEIISRRTQ RRLRRIKESW GLQDLVNDFG  420
NLQLGPPMS                                                          429

SEQ ID NO: 30           moltype = AA  length = 429
FEATURE                 Location/Qualifiers
source                  1..429
                        mol_type = protein
                        organism = Rattus rattus
SEQUENCE: 30
MGPFCLGCSH RKCYSPIRNL ISQETFKFHF KNLRYAIDRK DTFLCYEVTR KDCDSPVSLH  60
HGVFKNKDNI HAEICFLYWF HDKVLKVLSP REEFKITWYM SWSPCFECAE QVLRFLATHH  120
NLSLDIFSSR LYNIRDPENQ QNLCRLVQEG AQVAAMDLYE FKKCWKKFVD NGGRRFRPWK  180
KLLTNFRYQD SKLQEILRPC YIPVPSSSSS TLSNICLTKG LPETRFCVER RRVHLLSEEE  240
FYSQFYNQRV KHLCYYHGVK PYLCYQLEQF NGQAPLKGCL LSEKGKQHAE ILFLDKIRSM  300
ELSQVIITCY LTWSPCPNCA WQLAAFKRDR PDLILHIYTS RLYFHWKRPF QKGLCSLWQS  360
GILVDVMDLP QFTDCWTNFV NPKRPFWPWK GLEIISRRTQ RRLHRIKESW GLQDLVNDFG  420
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NLQLGPPMS                                                          429

SEQ ID NO: 31          moltype = AA  length = 370
FEATURE                Location/Qualifiers
REGION                 1..370
                       note = Synthetic
source                 1..370
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 31
MVEPMDPRTF VSNFNNRPIL SGLNTVWLCC EVKTKDPSGP PLDAKIFQGK VYSKAKYHPE  60
MRFLRWFHKW RQLHHDQEYK VTWYVSWSPC TRCANSVATF LAKDPKVTLT IFVARLYYFW  120
KPDYQQALRI LCQKRGGPHA TMKIMNYNEF QDCWNKFVDG RGKPFKPRNN LPKHYTLLQA  180
TLGELLRHLM DPGTFTSNFN NKPWVSGQHE TYLCYKVERL HNDTWVPLNQ HRGFLRNQAP  240
NIHGFPKGRH AELCFLDLIP FWKLDGQQYR VTCFTSWSPC FSCAQEMAKF ISNNEHVSLC  300
IFAARIYDDQ GRYQEGLRAL HRDGAKIAMM NYSEFEYCWD TFVDRQGRPF QPWDGLDEHS  360
QALSGRLRAI                                                         370

SEQ ID NO: 32          moltype = AA  length = 384
FEATURE                Location/Qualifiers
REGION                 1..384
                       note = Synthetic
source                 1..384
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 32
MKPHFRNPVE RMYQDTFSDN FYNRPILSHR NTVWLCYEVK TKGPSRPPLD AKIFRGQVYS  60
KLKYHPEMRF FHWFSKWRKL HRDQEYEVTW YISWSPCTKC TRDVATFLAE DPKVTLTIFV  120
ARLYYFWDPD YQEALRSLCQ KRDGPRATMK IMNYDEFQHC WSKFVYSQRE LFEPWNNLPK  180
YYILLHIMLG EILRHSMDPP TFTSNFNNEL WVRGRHETYL CYEVERLHND TWVLLNQRRG  240
FLCNQAPHKH GFLEGRHAEL CFLDVIPFWK LDLHQDYRVT CFTSWSPCFS CAQEMAKFIS  300
NNKHVSLCIF AARIYDDQGR CQEGLRTLAK AGAKISIMTY SEFKHCWDTF VDHQGCPFQP  360
WDGLEEHSQA LSGRLRAILQ NQGN                                         384

SEQ ID NO: 33          moltype = AA  length = 377
FEATURE                Location/Qualifiers
REGION                 1..377
                       note = Synthetic
source                 1..377
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 33
MNPQIRNMVE QMEPDIFVYY FNNRPILSGR NTVWLCYEVK TKDPSGPPLD ANIFQGKLYP  60
EAKDHPEMKF LHWFRKWRQL HRDQEYEVTW YVSWSPCTRC ANSVATFLAE DPKVTLTIFV  120
ARLYYFWKPD YQQALRILCQ ERGGPHATMK IMNYNEFQHC WNEFVDGQGK PFKPRKNLPK  180
HYTLLHATLG ELLRHVMDPG TFTSNFNNKP WVSGQRETYL CYKVERSHND TWVLLNQHRG  240
FLRNQAPDRH GFPKGRHAEL CFLDLIPFWK LDDQQYRVTC FTSWSPCFSC AQKMAKFISN  300
NKHVSLCIFA ARIYDDQGRC QEGLRTLHRD GAKIAVMNYS EFEYCWDTFV DRQGRPFQPW  360
DGLDEHSQAL SGRLRAI                                                 377

SEQ ID NO: 34          moltype = AA  length = 384
FEATURE                Location/Qualifiers
source                 1..384
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 34
MKPHFRNTVE RMYRDTFSYN FYNRPILSRR NTVWLCYEVK TKGPSRPPLD AKIFRGQVYS  60
ELKYHPEMRF FHWFSKWRKL HRDQEYEVTW YISWSPCTKC TRDMATFLAE DPKVTLTIFV  120
ARLYYFWDPD YQEALRSLCQ KRDGPRATMK IMNYDEFQHC WSKFVYSQRE LFEPWNNLPK  180
YYILLHIMLG EILRHSMDPP TFTFNFNNEP WVRGRHETYL CYEVERMHND TWVLLNQRRG  240
FLCNQAPHKH GFLEGRHAEL CFLDVIPFWK LDLDQDYRVT CFTSWSPCFS CAQEMAKFIS  300
KNKHVSLCIF TARIYDDQGR CQEGLRTLAE AGAKISIMTY SEFKHCWDTF VDHQGCPFQP  360
WDGLDEHSQD LSGRLRAILQ NQEN                                         384

SEQ ID NO: 35          moltype = AA  length = 373
FEATURE                Location/Qualifiers
source                 1..373
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 35
MKPHFRNTVE RMYRDTFSYN FYNRPILSRR NTVWLCYEVK TKGPSRPRLD AKIFRGQVYS  60
QPEHHAEMCF LSWFCGNQLP AYKCFQITWF VSWTPCPDCV AKLAEFLAEH PNVTLTISAA  120
RLYYYWERDY RRALCRLSQA GARVKIMDDE EFAYCWENFV YSEGQPFMPW YKFDDNYAFL  180
HRTLKEILRN PMEAMYPHIF YFHFKNLRKA YGRNESWLCF TMEVVKHHSP VSWKRGVFRN  240
QVDPETHCHA ERCFLSWFCD DILSPNTNYE VTWYTSWSPC PECAGEVAEF LARHSNVNLT  300
IFTARLYYFW DTDYQEGLRS LSQEGASVEI MGYKDFKYCW ENFVYNDDEP FKPWKGLKYN  360
```

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FLFLDSKLQE ILE                                                    373

SEQ ID NO: 36          moltype = AA  length = 382
FEATURE                Location/Qualifiers
source                 1..382
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 36
MNPQIRNPME RMYRDTFYDN FENEPILYGR SYTWLCYEVK IKRGRSNLLW DTGVFRGQVY  60
FKPQYHAEMC FLSWFCGNQL PAYKCFQITW FVSWTPCPDC VAKLAEFLSE HPNVTLTISA  120
ARLYYYWERD YRRALCRLSQ AGARVTIMDY EEFAYCWENF VYNEGQQFMP WYKFDENYAF  180
LHRTLKEILR YLMDPDTFTF NFNNDPLVLR RRQTYLCYEV ERLDNGTWVL MDQHMGFLCN  240
EAKNLLCGFY GRHAELRFLD LVPSLQLDPA QIYRVTWFIS WSPCFSWGCA GEVRAFLQEN  300
THVRLRIFAA RIYDYDPLYK EALQMLRDAG AQVSIMTYDE FEYCWDTFVY RQGCPFQPWD  360
GLEEHSQALS GRLRAILQNQ GN                                          382

SEQ ID NO: 37          moltype = AA  length = 190
FEATURE                Location/Qualifiers
source                 1..190
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 37
MNPQIRNPMK AMYPGTFYFQ FKNLWEANDR NETWLCFTVE GIKRRSVVSW KTGVFRNQVD  60
SETHCHAERC FLSWFCDDIL SPNTKYQVTW YTSWSPCPDC AGEVAEFLAR HSNVNLTIFT  120
ARLYYFQYPC YQEGLRSLSQ EGVAVEIMDY EDFKYCWENF VYNDNEPFKP WKGLKTNFRL  180
LKRRLRESLQ                                                        190

SEQ ID NO: 38          moltype = AA  length = 199
FEATURE                Location/Qualifiers
source                 1..199
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 38
MEASPASGPR HLMDPHIFTS NFNNGIGRHK TYLCYEVERL DNGTSVKMDQ HRGFLHNQAK  60
NLLCGFYGRH AELRFLDLVP SLQLDPAQIY RVTWFISWSP CFSWGCAGEV RAFLQENTHV  120
RLRIFAARIY DYDPLYKEAL QMLRDAGAQV SIMTYDEFKH CWDTFVDHQG CPFQPWDGLD  180
EHSQALSGRL RAILQNQGN                                              199

SEQ ID NO: 39          moltype = AA  length = 200
FEATURE                Location/Qualifiers
source                 1..200
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 39
MALLTAETFR LQFNNKRRLR RPYYPRKALL CYQLTPQNGS TPTRGYFENK KKCHAEICFI  60
NEIKSMGLDE TQCYQVTCYL TWSPCSSCAW ELVDFIKAHD HLNLGIFASR LYYHWCKPQQ  120
KGLRLLCGSQ VPVEVMGFPK FADCWENFVD HEKPLSFNPY KMLEELDKNS RAIKRRLERI  180
KIPGVRAQGR YMDILCDAEV                                             200

SEQ ID NO: 40          moltype = AA  length = 386
FEATURE                Location/Qualifiers
source                 1..386
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 40
MNPQIRNPME RMYRDTFYDN FENEPILYGR SYTWLCYEVK IKRGRSNLLW DTGVFRGPVL  60
PKRQSNHRQE VYFRFENHAE MCFLSWFCGN RLPANRRFQI TWFVSWNPCL PCVVKVTKFL  120
AEHPNVTLTI SAARLYYYRD RDWRWVLLRL HKAGARVKIM DYEDFAYCWE NFVCNEGQPF  180
MPWYKFDDNY ASLHRTLKEI LRNPMEAMYP HIFYFHFKNL LKACGRNESW LCFTMEVTKH  240
HSAVFRKRGV FRNQVDPETH CHAERCFLSW FCDDILSPNT NYEVTWYTSW SPCPECAGEV  300
AEFLARHSNV NLTIFTARLC YFWDTDYQEG LCSLSQEGAS VKIMGYKDFV SCWKNFVYSD  360
DEPFKPWKGL QTNFRLLKRR LREILQ                                      386

SEQ ID NO: 41          moltype = AA  length = 236
FEATURE                Location/Qualifiers
source                 1..236
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 41
MTSEKGPSTG DPTLRRRIEP WEFDVFYDPR ELRKEACLLY EIKWGMSRKI WRSSGKNTTN  60
HVEVNFIKKF TSERDFHPSM SCSITWFLSW SPCWECSQAI REFLSRHPGV TLVIYVARLF  120
WHMDQQNRQG LRDLVNSGVT IQIMRASEYY HCWRNFVNYP PGDEAHWPQY PPLWMMLYAL  180
ELHCIILSLP PCLKISRRWQ NHLTFFRLHL QNCHYQTIPP HILLATGLIH PSVAWR      236

SEQ ID NO: 42          moltype = AA  length = 229
FEATURE                Location/Qualifiers
```

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```
source                  1..229
                        mol_type = protein
                        organism = Mus musculus
SEQUENCE: 42
MSSETGPVAV DPTLRRRIEP HEFEVFFDPR ELRKETCLLY EINWGGRHSV WRHTSQNTSN  60
HVEVNFLEKF TTERYFRPNT RCSITWFLSW SPCGECSRAI TEFLSRHPYV TLFIYIARLY  120
HHTDQRNRQG LRDLISSGVT IQIMTEQEYC YCWRNFVNYP PSNEAYWPRY PHLWVKLYVL  180
ELYCIILGLP PCLKILRRKQ PQLTFFTITL QTCHYQRIPP HLLWATGLK              229

SEQ ID NO: 43           moltype = AA  length = 229
FEATURE                 Location/Qualifiers
source                  1..229
                        mol_type = protein
                        organism = Rattus rattus
SEQUENCE: 43
MSSETGPVAV DPTLRRRIEP HEFEVFFDPR ELRKETCLLY EINWGGRHSI WRHTSQNTNK  60
HVEVNFIEKF TTERYFCPNT RCSITWFLSW SPCGECSRAI TEFLSRYPHV TLFIYIARLY  120
HHADPRNRQG LRDLISSGVT IQIMTEQESG YCWRNFVNYS PSNEAHWPRY PHLWVRLYVL  180
ELYCIILGLP PCLNILRRKQ PQLTFFTIAL QSCHYQRLPP HILWATGLK              229

SEQ ID NO: 44           moltype = AA  length = 239
FEATURE                 Location/Qualifiers
source                  1..239
                        mol_type = protein
                        organism = Rattus rattus
SEQUENCE: 44
MAVGSKPKAA LVGPHWERER IWCFLCSTGL GTQQTGQTSR WLRPAATQDP VSPPRSLLMK  60
QRKFLYHFKN VRWAKGRHET YLCYVVKRRD SATSFSLDFG YLRNKSGCHV ELLFLRYISD  120
WDLDPGRCYR VTWFTSWSPC YDCARHVADF LRGNPNLSLR IFTARLTGWG ALPAGLMSPA  180
RPSDYFYCWN TFVENHERTF KAWEGLHENS VRLSRRLRRI LLPLYEVDDL RDAFRTLGL   239

SEQ ID NO: 45           moltype = AA  length = 190
FEATURE                 Location/Qualifiers
source                  1..190
                        mol_type = protein
                        organism = Gorilla gorilla
SEQUENCE: 45
MNPQIRNPMK AMYPGTFYFQ FKNLWEANDR NETWLCFTVE GIKRRSVVSW KTGVFRNQVD  60
SETHCHAERC FLSWFCDDIL SPNTNYQVTW YTSWSPCPEC AGEVAEFLAR HSNVNLTIFT  120
ARLYYFQDTD YQEGLRSLSQ EGVAVKIMDY KDFKYCWENF VYNDDEPFKP WKGLKYNFRF  180
LKRRLQEILE                                                         190

SEQ ID NO: 46           moltype = AA  length = 202
FEATURE                 Location/Qualifiers
REGION                  1..202
                        note = Synthetic
source                  1..202
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 46
MDGSPASRPR HLMDPNTFTF NFNNDLSVRG RHQTYLCYEV ERLDNGTWVP MDERRGFLCN  60
KAKNVPCGDY GCHVELRFLC EVPSWQLDPA QTYRVTWFIS WSPCFRRGCA GQVRVFLQEN  120
KHVRLRIFAA RIYDYDPLYQ EALRTLRDAG AQVSIMTYEE FKHCWDTFVD RQGRPFQPWD  180
GLDEHSQALS GRLRAILQNQ GN                                           202

SEQ ID NO: 47           moltype = AA  length = 185
FEATURE                 Location/Qualifiers
source                  1..185
                        mol_type = protein
                        note = Bos bovis
                        organism = unidentified
SEQUENCE: 47
MDEYTFTENF NNQGWPSKTY LCYEMERLDG DATIPLDEYK GFVRNKGLDQ PEKPCHAELY  60
FLGKIHSWNL DRNQHYRLTC FISWSPCYDC AQKLTTFLKE NHHISLHILA SRIYTHNRFG  120
CHQSGLCELQ AAGARITIMT FEDFKHCWET FVDHKGKPFQ PWEGLNVKSQ ALCTELQAIL  180
KTQQN                                                              185

SEQ ID NO: 48           moltype = AA  length = 396
FEATURE                 Location/Qualifiers
source                  1..396
                        mol_type = protein
                        organism = Rattus rattus
SEQUENCE: 48
MQPQGLGPNA GMGPVCLGCS HRRPYSPIRN PLKKLYQQTF YFHFKNVRYA WGRKNNFLCY  60
EVNGMDCALP VPLRQGVFRK QGHIHAELCF IYWFHDKVLR VLSPMEEFKV TWYMSWSPCS  120
KCAEQVARFL AAHRNLSLAI FSSRLYYYLR NPNYQQKLCR LIQEGVHVAA MDLPEFKKCW  180
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NKFVDNDGQP FRPWMRLRIN FSFYDCKLQE IFSRMNLLRE DVFYLQFNNS HRVKPVQNRY  240
YRRKSYLCYQ LERANGQEPL KGYLLYKKGE QHVEILFLEK MRSMELSQVR ITCYLTWSPC  300
PNCARQLAAF KKDHPDLILR IYTSRLYFYW RKKFQKGLCT LWRSGIHVDV MDLPQFADCW  360
TNFVNPQRPF RPWNELEKNS WRIQRRLRRI KESWGL                            396

SEQ ID NO: 49          moltype = AA  length = 227
FEATURE                Location/Qualifiers
source                 1..227
                       mol_type = protein
                       note = Bos bovis
                       organism = unidentified
SEQUENCE: 49
DGWEVAFRSG TVLKAGVLGV SMTEGWAGSG HPGQGACVWT PGTRNTMNLL REVLFKQQFG  60
NQPRVPAPYY RRKTYLCYQL KQRNDLTLDR GCFRNKKQRH AEIRFIDKIN SLDLNPSQSY  120
KIICYITWSP CPNCANELVN FITRNNHLKL EIFASRLYFH WIKSFKMGLQ DLQNAGISVA  180
VMTHTEFEDC WEQFVDNQSR PFQPWDKLEQ YSASIRRRLQ RILTAPI                227

SEQ ID NO: 50          moltype = AA  length = 490
FEATURE                Location/Qualifiers
REGION                 1..490
                       note = Synthetic
source                 1..490
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 50
MNPQIRNPME WMYQRTFYYN FENEPILYGR SYTWLCYEVK IRRGHSNLLW DTGVFRGQMY  60
SQPEHHAEMC FLSWFCGNQL SAYKCFQITW FVSWTPCPDC VAKLAKFLAE HPNVTLTISA  120
ARLYYYWERD YRRALCRLSQ AGARVKIMDD EEFAYCWENF VYNEGQPFMP WYKFDDNYAF  180
LHRTLKEIIR HLMDPDTFTF NFNNDPLVLR RHQTYLCYEV ERLDNGTWVL MDQHMGFLCN  240
EAKNLLCGFY GRHAELRFLD LVPSLQLDPA QIYRVTWFIS WSPCFSWGCA GQVRAFLQEN  300
THVRLRIFAA RIYDYDPLYK EALQMLRDAG AQVSIMTYDE FEYCWDTFVY RQGCPFQPWD  360
GLEEHSQALS GRLRAILQVR ASSLCMVPHR PPPPPQSPGP CLPLCSEPPL GSLLPTGRPA  420
PSLPFLLTAS FSFPPPASLP PLPSLSLSPG HLPVPSFHSL TSCSIQPPCS SRIRETEGWA  480
SVSKEGRDLG                                                         490

SEQ ID NO: 51          moltype = AA  length = 210
FEATURE                Location/Qualifiers
REGION                 1..210
                       note = Synthetic
source                 1..210
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 51
MALLTAKTFS LQFNNKRRVN KPYYPRKALL CYQLTPQNGS TPTRGHLKNK KKDHAEIRFI  60
NKIKSMGLDE TQCYQVTCYL TWSPCPSCAG ELVDFIKAHR HLNLRIFASR LYYHWRPNYQ  120
EGLLLLCGSQ VPVEVMGLPE FTDCWENFVD HKEPPSFNPS EKLEELDKNS QAIKRRLERI  180
KSRSVDVLEN GLRSLQLGPV TPSSSIRNSR                                   210

SEQ ID NO: 52          moltype = AA  length = 224
FEATURE                Location/Qualifiers
source                 1..224
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 52
MAQKEEAAVA TEAASQNGED LENLDDPEKL KELIELPPFE IVTGERLPAN FFKFQFRNVE  60
YSSGRNKTFL CYVVEAQGKG GQVQASRGYL EDEHAAAHAE EAFFNTILPA FDPALRYNVT  120
WYVSSSPCAA CADRIIKTLS KTKNLRLLIL VGRLFMWEEP EIQAALKKLK EAGCKLRIMK  180
PQDFEYVWQN FVEQEEGESK AFQPWEDIQE NFLYYEEKLA DILK                   224

SEQ ID NO: 53          moltype = AA  length = 224
FEATURE                Location/Qualifiers
source                 1..224
                       mol_type = protein
                       organism = Mus musculus
SEQUENCE: 53
MAQKEEAAEA AAPASQNGDD LENLEDPEKL KELIDLPPFE IVTGVRLPVN FFKFQFRNVE  60
YSSGRNKTFL CYVVEVQSKG GQAQATQGYL EDEHAGAHAE EAFFNTILPA FDPALKYNVT  120
WYVSSSPCAA CADRILKTLS KTKNLRLLIL VSRLFMWEEP EVQAALKKLK EAGCKLRIMK  180
PQDFEYIWQN FVEQEEGESK AFEPWEDIQE NFLYYEEKLA DILK                   224

SEQ ID NO: 54          moltype = AA  length = 224
FEATURE                Location/Qualifiers
source                 1..224
                       mol_type = protein
                       organism = Rattus rattus
SEQUENCE: 54
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MAQKEEAAEA AAPASQNGDD LENLEDPEKL KELIDLPPFE IVTGVRLPVN FFKFQFRNVE  60
YSSGRNKTFL CYVVEAQSKG GQVQATQGYL EDEHAGAHAE EAFFNTILPA FDPALKYNVT  120
WYVSSSPCAA CADRILKTLS KTKNLRLLIL VSRLFMWEEP EVQAALKKLK EAGCKLRIMK  180
PQDFEYLWQN FVEQEEGESK AFEPWEDIQE NFLYYEEKLA DILK                   224

SEQ ID NO: 55          moltype = AA  length = 224
FEATURE                Location/Qualifiers
source                 1..224
                       mol_type = protein
                       note = Bos bovis
                       organism = unidentified
SEQUENCE: 55
MAQKEEAAAA AEPASQNGEE VENLEDPEKL KELIELPPFE IVTGERLPAH YFKFQFRNVE  60
YSSGRNKTFL CYVVEAQSKG GQVQASRGYL EDEHATNHAE EAFFNSIMPT FDPALRYMVT  120
WYVSSSPCAA CADRIVKTLN KTKNLRLLIL VGRLFMWEEP EIQAALRKLK EAGCRLRIMK  180
PQDFEYIWQN FVEQEEGESK AFEPWEDIQE NFLYYEEKLA DILK                   224

SEQ ID NO: 56          moltype = AA  length = 208
FEATURE                Location/Qualifiers
source                 1..208
                       mol_type = protein
                       organism = Petromyzon marinus
SEQUENCE: 56
MTDAEYVRIH EKLDIYTFKK QFFNNKKSVS HRCYVLFELK RRGERRACFW GYAVNKPQSG  60
TERGIHAEIF SIRKVEEYLR DNPGQFTINW YSSWSPCADC AEKILEWYNQ ELRGNGHTLK  120
IWACKLYYEK NARNQIGLWN LRDNGVGLNV MVSEHYQCCR KIFIQSSHNQ LNENRWLEKT  180
LKRAEKRRSE LSIMIQVKIL HTTKSPAV                                     208

SEQ ID NO: 57          moltype = AA  length = 384
FEATURE                Location/Qualifiers
source                 1..384
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 57
MKPHFRNTVE RMYRDTFSYN FYNRPILSRR NTVWLCYEVK TKGPSRPPLD AKIFRGQVYS  60
ELKYHPEMRF FHWFSKWRKL HRDQEYEVTW YISWSPCTKC TRDMATFLAE DPKVTLTIFV  120
ARLYYFWDPD YQEALRSLCQ KRDGPRATMK IMNYDEFQHC WSKFVYSQRE LFEPWNNLPK  180
YYILLHIMLG EILRHSMDPP TFTFNFNNEP WVRGRHETYL CYEVERMHND TWVLLNQRRG  240
FLCNQAPHKH GFLEGRHAEL CFLDVIPFWK LDLDQDYRVT CFTSWSPCFS CAQEMAKFIS  300
KNKHVSLCIF TARIYRRQGR CQEGLRTLAE AGAKISIMTY SEFKHCWDTF VDHQGCPFQP  360
WDGLDEHSQD LSGRLRAILQ NQEN                                         384

SEQ ID NO: 58          moltype = AA  length = 184
FEATURE                Location/Qualifiers
source                 1..184
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 58
MDPPTFTFNF NNEPWVRGRH ETYLCYEVER MHNDTWVLLN QRRGFLCNQA PHKHGFLEGR  60
HAELCFLDVI PFWKLDLDQD YRVTCFTSWS PCFSCAQEMA KFISKNKHVS LCIFTARIYD  120
DQGRCQEGLR TLAEAGAKIS IMTYSEFKHC WDTFVDHQGC PFQPWDGLDE HSQDLSGRLR  180
AILQ                                                               184

SEQ ID NO: 59          moltype = AA  length = 184
FEATURE                Location/Qualifiers
source                 1..184
                       mol_type = protein
                       organism = Homo sapiens
SEQUENCE: 59
MDPPTFTFNF NNEPWVRGRH ETYLCYEVER MHNDTWVLLN QRRGFLCNQA PHKHGFLEGR  60
HAELCFLDVI PFWKLDLDQD YRVTCFTSWS PCFSCAQEMA KFISKNKHVS LCIFTARIYR  120
RQGRCQEGLR TLAEAGAKIS IMTYSEFKHC WDTFVDHQGC PFQPWDGLDE HSQDLSGRLR  180
AILQ                                                               184

SEQ ID NO: 60          moltype = AA  length = 84
FEATURE                Location/Qualifiers
REGION                 1..84
                       note = Synthetic
source                 1..84
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 60
MTNLSDIIEK ETGKQLVIQE SILMLPEEVE EVIGNKPESD ILVHTAYDES TDENVMLLTS  60
DAPEYKPWAL VIQDSNGENK IKML                                         84

SEQ ID NO: 61          moltype = AA  length = 181
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FEATURE                 Location/Qualifiers
REGION                  1..181
                        note = Erwinia tasmaniensis
source                  1..181
                        mol_type = protein
                        organism = unidentified
SEQUENCE: 61
MASRGVNKVI LVGNLGQDPE VRYMPNGGAV ANITLATSES WRDKQTGETK EKTEWHRVVL  60
FGKLAEVAGE YLRKGSQVYI EGALQTRKWT DQAGVEKYTT EVVVNVGGTM QMLGGRSQGG  120
GASAGGQNGG SNNGWGQPQQ PQGGNQFSGG AQQQARPQQQ PQQNNAPANN EPPIDFDDDI  180
P                                                                  181

SEQ ID NO: 62           moltype = AA  length = 209
FEATURE                 Location/Qualifiers
REGION                  1..209
                        note = Synthetic
source                  1..209
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 62
MAGAQDFVPH TADLAELAAA AGECRGCGLY RDATQAVFGA GGRSARIMMI GEQPGDKEDL  60
AGLPFVGPAG RLLDRALEAA DIDRDALYVT NAVKHFKFTR AAGGKRRIHK TPSRTEVVAC  120
RPWLIAEMTS VEPDVVVLLG ATAAKALLGN DFRVTQHRGE VLHVDDVPGD PALVATVHPS  180
SLLRGPKEER ESAFAGLVDD LRVAADVRP                                    209

SEQ ID NO: 63           moltype = AA  length = 313
FEATURE                 Location/Qualifiers
source                  1..313
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 63
MIGQKTLYSF FSPSPARKRH APSPEPAVQG TGVAGVPEES GDAAAIPAKK APAGQEEPGT  60
PPSSPLSAEQ LDRIQRNKAA ALLRLAARNV PVGFGESWKK HLSGEFGKPY FIKLMGFVAE  120
ERKHYTVYPP PHQVFTWTQM CDIKDVKVVI LGQEPYHGPN QAHGLCFSVQ RPVPPPPSLE  180
NIYKELSTDI EDFVHPGHGD LSGWAKQGVL LLNAVLTVRA HQANSHKERG WEQFTDAVVS  240
WLNQNSNGLV FLLWGSYAQK KGSAIDRKRH HVLQTAHPSP LSVYRGFFGC RHFSKTNELL  300
QKSGKKPIDW KEL                                                     313

SEQ ID NO: 64           moltype = AA  length = 148
FEATURE                 Location/Qualifiers
source                  1..148
                        mol_type = protein
                        organism = Homo sapiens
SEQUENCE: 64
MFRRPVLQVL RQFVRHESET TTSLVLERSL NRVHLLGRVG QDPVLRQVEG KNPVTIFSLA  60
TNEMWRSGDS EVYQLGDVSQ KTTWHRISVF RPGLRDVAYQ YVKKGSRIYL EGKIDYGEYM  120
DKNNVRRQAT TIIADNIIFL SDQTKEKE                                     148

SEQ ID NO: 65           moltype = AA  length = 388
FEATURE                 Location/Qualifiers
source                  1..388
                        mol_type = protein
                        organism = Mus musculus
SEQUENCE: 65
MFAKGKGSAV PSDGQAREKL ALYVYEYLLH VGAQKSAQTF LSEIRWEKNI TLGEPPGFLH  60
SWWCVFWDLY CAAPERRDTC EHSSEAKAFH DYSAAAAPSP VLGNIPPNDG MPGGPIPPGF  120
FQGPPGSQPS PHAQPPPHNP SSMMGPHSQP FMSPRYAGGP RPPIRMGNQP PGGVPGTQPL  180
LPNSMDPTRQ QGHPNMGGSM QRMNPPRGMG PMGPGPQNYG SGMRPPPNSL GPAMPGINMG  240
PGAGRPWPNP NSANSIPYSS SSPGTYVGPP GGGGPPGTPI MPSPADSTNS SDNIYTMINP  300
VPPGGSRSNF PMGPGSDGPM GGMGGMEPHH MNGSLGSGDI DGLPKNSPNN ISGISNPPGT  360
PRDDGELGGN FLHSFQNDNY SPSMTMSV                                     388

SEQ ID NO: 66           moltype = AA  length = 616
FEATURE                 Location/Qualifiers
REGION                  1..616
                        note = Synthetic
source                  1..616
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 66
MVGQLSEGAI AAIMQKGDTN IKPILQVINI RPITTGNSPP RYRLLMSDGL NTLSSFMLAT  60
QLNPLVEEEQ LSSNCVCQIH RFIVNTLKDG RRVVILMELE VLKSAEAVGV KIGNPVPYNE  120
GLGQPQVAPP APAASPAASS RPQPQNGSSG MGSTVSKAYG ASKTFGKAAG PSLSHTSGGT  180
QSKVVPIASL TPYQSKWTIC ARVTNKSQIR TWSNSRGEGK LFSLELVDES GEIRATAFNE  240
QVDKFFPLIE VNKVYYFSKG TLKIANKQFT AVKNDYEMTF NNETSVMPCE DDHHLPTVQF  300
DFTGIDDLEN KSKDSLVDII GICKSYEDAT KITVRSNNRE VAKRNIYLMD TSGKVVTATL  360
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WGEDADKFDG SRQPVLAIKG ARVSDFGGRS LSVLSSSTII ANPDIPEAYK LRGWFDAEGQ  420
ALDGVSISDL KSGGVGGSNT NWKTLYEVKS ENLGQGDKPD YFSSVATVVY LRKENCMYQA  480
CPTQDCNKKV IDQQNGLYRC EKCDTEFPNF KYRMILSVNI ADFQENQWVT CFQESAEAIL  540
GQNAAYLGEL KDKNEQAFEE VFQNANFRSF IFRVRVKVET YNDESRIKAT VMDVKPVDYR  600
EYGRRLVMSI RRSALM                                                  616

SEQ ID NO: 67          moltype = AA  length = 270
FEATURE                Location/Qualifiers
REGION                 1..270
                       note = Synthetic
source                 1..270
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 67
MWNSGFESYG SSSYGGAGGY TQSPGGFGSP APSQAEKKSR ARAQHIVPCT ISQLLSATLV  60
DEVFRIGNVE ISQVTIVGII RHAEKAPTNI VYKIDDMTAA PMDVRQWVDT DDTSSENTVV  120
PPETYVKVAG HLRSFQNKKS LVAFKIMPLE DMNEFTTHIL EVINAHMVLS KANSQPSAGR  180
APISNPGMSE AGNFGGNSFM PANGLTVAQN QVLNLIKACP RPEGLNFQDL KNQLKHMSVS  240
SIKQAVDFLS NEGHIYSTVD DDHFKSTDAE                                   270

SEQ ID NO: 68          moltype = AA  length = 121
FEATURE                Location/Qualifiers
REGION                 1..121
                       note = Synthetic
source                 1..121
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 68
MVDMMDLPRS RINAGMLAQF IDKPVCFVGR LEKIHPTGKM FILSDGEGKN GTIELMEPLD  60
EEISGIVEVV GRVTAKATIL CTSYVQFKED SHPFDLGLYN EAVKIIHDFP QFYPLGIVQH  120
D                                                                  121

SEQ ID NO: 69          moltype = AA  length = 172
FEATURE                Location/Qualifiers
source                 1..172
                       mol_type = protein
                       organism = Bacillus subtilis
SEQUENCE: 69
MLNRVVLVGR LTKDPELRYT PNGAAVATFT LAVNRTFTNQ SGEREADFIN CVTWRRQAEN  60
VANFLKKGSL AGVDGRLQTR NYENQQGQRV FVTEVQAESV QFLEPKNGGG SGSGGYNEGN  120
SGGGQYFGGG QNDNPFGGNQ NNQRRNQGNS FNDDPFANDG KPIDISDDDL PF          172

SEQ ID NO: 70          moltype = AA  length = 199
FEATURE                Location/Qualifiers
source                 1..199
                       mol_type = protein
                       note = Streptomyces coelicolor
                       organism = unidentified
SEQUENCE: 70
MAGETVITVV GNLVDDPELR FTPSGAAVAK FRVASTPRTF DRQTNEWKDG ESLFLTCSVW  60
RQAAENVAES LQRGMRVIVQ GRLKQRSYED REGVKRTVYE LDVDEVGASL RSATAKVTKT  120
SGQGRGGQGG YGGGGGGQGG GGWGGGPGGG QQGGGAPADD PWATGGAPAG GQQGGGGQGG  180
GGWGGGSGGG GGYSDEPPF                                               199

SEQ ID NO: 71          moltype = AA  length = 156
FEATURE                Location/Qualifiers
source                 1..156
                       mol_type = protein
                       organism = Streptococcus pneumoniae
SEQUENCE: 71
MINNVVLVGR MTRDAELRYT PSNVAVATFT LAVNRTFKSQ NGEREADFIN VVMWRQQAEN  60
LANWAKKGSL IGVTGRIQTR SYDNQQGQRV YVTEVVAENF QMLESRSVRE GHTGGAYSAP  120
TANYSAPTNS VPDFSRNENP FGATNPLDIS DDDLPF                            156

SEQ ID NO: 72          moltype = AA  length = 1196
FEATURE                Location/Qualifiers
REGION                 1..1196
                       note = Synthetic
source                 1..1196
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 72
METKPKTATT IKVPPGPLGY VYARACPSEG IELLALLSAR SGDSDVAVAP LVVGLTVESG  60
FEANVAVVVG SRTTGLGGTA VSLKLTPSHY SSSVYVFHGG RHLDPSTQAP NLTRLCERAR  120
RHFGFSDYTP RPGDLKHETT GEALCERLGL DPDRALLYLV VTEGFKEAVC INNTFLHLGG  180
SDKVTIGGAE VHRIPVYPLQ LFMPDFSRVI AEPFNANHRS IGENFTYPLP FFNRPLNRLL  240
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FEAVVGPAAV ALRCRNVDAV ARAAAHLAFD ENHEGAALPA DITFTAFEAS QGKTPRGGRD  300
GGGKGPAGGF EQRLASVMAG DAALALESIV SMAVFDEPPT DISAWPLFEG QDTAAARANA  360
VGAYLARAAG LVGAMVFSTN SALHLTEVDD AGPADPKDHS KPSFYRFFLV PGTHVAANPQ  420
VDREGHVVPG FEGRPTAPLV GGTQEFAGEH LAMLCGFSPA LLAKMLFYLE RCDGGVIVGR  480
QEMDVFRYVA DSNQTDVPCN LCTFDTRHAC VHTTLMRLRA RHPKFASAAR GAIGVFGTMN  540
SMYSDCDVLG NYAAFSALKR ADGSETARTI MQETYRAATE RVMAELETLQ YVDQAVPTAM  600
GRLETIITNR EALHTVVNNV RQVVDREVEQ LMRNLVEGRN FKFRDGLGEA NHAMSLTLDP  660
YACGPCPLLQ LLGRRSNLAV YQDLALSQCH GVFAGQSVEG RNFRNQFQPV LRRRVMDMFN  720
NGFLSAKTLT VALSEGAAIC APSLTAGQTA PAESSFEGDV ARVTLGFPKE LRVKSRVLFA  780
GASANASEAA KARVASLQSA YQKPDKRVDI LLGPLGFLLK QFHAAIFPNG KPPGSNQPNP  840
QWFWTALQRN QLPARLLSRE DIETIAFIKK FSLDYGAINF INLAPNNVSE LAMYYMANQI  900
LRYCDHSTYF INTLTAIIAG SRRPPSVQAA AAWSAQGGAG LEAGARALMD AVDAHPGAWT  960
SMFASCNLLR PVMAARPMVV LGLSISKYYG MAGNDRVFQA GNWASLMGGK NACPLLIFDR  1020
TRKFVLACPR AGFVCAASSL GGGAHESSLC EQLRGIISEG GAAVASSVFV ATVKSLGPRT  1080
QQLQIEDWLA LLEDEYLSEE MMELTARALE RGNGEWSTDA ALEVAHEAEA LVSQLGNAGE  1140
VFNFGDFGCE DDNATPFGGP GAPGPAFAGR KRAFHGDDPF GEGPPDKKGD LTLDML      1196

SEQ ID NO: 73          moltype = AA  length = 124
FEATURE                Location/Qualifiers
REGION                 1..124
                       note = Synthetic
source                 1..124
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 73
MENTNIVKAT FDTETLEGQI KIFNAQTGGG QSFKNLPDGT IIEANAIAQY KQVSDTYGDA  60
KEETVTTIFA ADGSLYSAIS KTVAEAASDL IDLVTRHKLE TFKVKVVQGT SSKGNVFFSL  120
QLSL                                                               124

SEQ ID NO: 74          moltype = AA  length = 162
FEATURE                Location/Qualifiers
REGION                 1..162
                       note = Synthetic
source                 1..162
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 74
MASVNKVILV GNLGADPETR YLPSGDAISN IRLATTDRYK DKASGEMKES TEWHRVSFFG  60
RLAEIVDEYL RKGAPVYIEG RIRTRKWQDN AGQDRYTTEI VAEKMQMLGD RRDGGERQQR  120
APQQQQQRTQ RNGYADATGR AQPSQRPAAG GGFDEMDDDI PF                     162

SEQ ID NO: 75          moltype = AA  length = 7
FEATURE                Location/Qualifiers
REGION                 1..7
                       note = Synthetic
source                 1..7
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 75
PKKKRKV                                                            7

SEQ ID NO: 76          moltype = AA  length = 30
FEATURE                Location/Qualifiers
REGION                 1..30
                       note = Synthetic
source                 1..30
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 76
MDSLLMNRRK FLYQFKNVRW AKGRRETYLC                                   30

SEQ ID NO: 77          moltype = RNA  length = 123
FEATURE                Location/Qualifiers
source                 1..123
                       mol_type = genomic RNA
                       note = Bacillus thermoamyloliquefaciens
                       organism = unidentified
SEQUENCE: 77
gtcatagttc ccctgagaaa tcagggttac tatgataagg gctttctgcc taaggcagac  60
tgacccgcgg cgttggggat cgcctgtcgc ccgcttttgg cgggcattcc ccatcctttt  120
ttt                                                                123

SEQ ID NO: 78          moltype = RNA  length = 87
FEATURE                Location/Qualifiers
misc_feature           1..87
                       note = Synthetic
```

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```
source                  1..87
                        mol_type = other RNA
                        organism = synthetic construct
SEQUENCE: 78
gcaagccccg agaaacaggg acagaaaggg ccgccaaggc agacgacccg cggcggggga  60
cgccgcgccc gcggcgggca ccccacc                                      87

SEQ ID NO: 79           moltype = AA  length = 8
FEATURE                 Location/Qualifiers
REGION                  1..8
                        note = Synthetic
source                  1..8
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 79
NNNNCRAA                                                           8

SEQ ID NO: 80           moltype = AA  length = 1429
FEATURE                 Location/Qualifiers
REGION                  1..1429
                        note = Synthetic
source                  1..1429
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 80
MSSETGPVAV DPTLRRRIEP HEFEVFFDPR ELRKETCLLY EINWGGRHSI WRHTSQNTNK  60
HVEVNFIEKF TTERYFCPNT RCSITWFLSW SPCGECSRAI TEFLSRYPHV TLFIYIARLY  120
HHADPRNRQG LRDLISSGVT IQIMTEQESG YCWRNFVNYS PSNEAHWPRY PHLWVRLYVL  180
ELYCIILGLP PCLNILRRKQ PQLTFFTIAL QSCHYQRLPP HILWATGLKS GSETPGTSES  240
ATPESRYKIG LAIGITSVGW AVMNLDIPRI EDLGVRIFDR AENPQTGESL ALPRRLARSA  300
RRRLRRRKHR LERIRRLVIR EGILTKEELD KLFEEKHEID VWQLRVEALD RKLNNDELAR  360
VLLHLAKRRG FKSNRKSERS NKENSTMLKH IEENRAILSS YRTVGEMIVK DPKFALHKRN  420
KGENYTNTIA RDDLEREIRL IFSKQREFGN MSCTEEFENE YITIWASQRP VASKDDIEKK  480
VGFCTFEPKE KRAPKATYTF QSFIAWEHIN KLRLISPSGA RGLTDEERRL LYEQAFQKNK  540
ITYHDIRTLL HLPDDTYFKG IVYDRGESRK QNENIRFLEL DAYHQIRKAV DKVYGKGKSS  600
SFLPIDFDTF GYALTLFKDD ADIHSYLRNE YEQNGKRMPN LANKVYDNEL IEELLNLSFT  660
KFGHLSLKAL RSILPYMEQG EVYSSACERA GYTFTGPKKK QKTMLLPNIP PIANPVVMRA  720
LTQARKVVNA IIKKYGSPVS IHIELARDLS QTFDERRKTK KEQDENRKKN ETAIRQLMEY  780
GLTLNPTGHD IVKFKLWSEQ NGRCAYSLQP IEIERLLEPG YVEVDHVIPY SRSLDDSYTN  840
KVLVLTRENR EKGNRIPAEY LGVGTERWQQ FETFVLTNKQ FSKKKRDRLL RLHYDENEET  900
EFKNRNLNDT RYISRFFANF IREHLKFAES DDKQKVYTVN GRVTAHLRSR WEFNKNREES  960
DLHHAVDAVI VACTTPSDIA KVTAFYQRRE QNKELAKKTE PHFPQPWPHF ADELRARLSK  1020
HPKESIKALN LGNYDDQKLE SLQPVFVSRM PKRSVTGAAH QETLRRYVGI DERSGKIQTV  1080
VKTKLSEIKL DASGHFPMYG KESDPRTYEA IRQRLLEHNN DPKKAFQEPL YKPKKNGEPG  1140
PVIRTVKIID TKNQVIPLND GKTVAYNSNI VRVDVFEKDG KYYCVPVYTM DIMKGILPNK  1200
AIEPNKPYSE WKEMTEDYTF RFSLYPNDLI RIELPREKTV KTAAGEEINV KDVFVYYKTI  1260
DSANGGLELI SHDHRFSLRG VGSRTLKRFE KYQVDVLGNI YKVRGEKRVG LASSAHSKPG  1320
KTIRPLQSTR DSGGSTNLSD IIEKETGKQL VIQESILMLP EEVEEVIGNK PESDILVHTA  1380
YDESTDENVM LLTSDAPEYK PWALVIQDSN GENKIKMLSG GSPKKKRKV              1429

SEQ ID NO: 81           moltype = AA  length = 1544
FEATURE                 Location/Qualifiers
REGION                  1..1544
                        note = Synthetic
source                  1..1544
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 81
MSSETGPVAV DPTLRRRIEP HEFEVFFDPR ELRKETCLLY EINWGGRHSI WRHTSQNTNK  60
HVEVNFIEKF TTERYFCPNT RCSITWFLSW SPCGECSRAI TEFLSRYPHV TLFIYIARLY  120
HHADPRNRQG LRDLISSGVT IQIMTEQESG YCWRNFVNYS PSNEAHWPRY PHLWVRLYVL  180
ELYCIILGLP PCLNILRRKQ PQLTFFTIAL QSCHYQRLPP HILWATGLKS GGSSGGSSGS  240
ETPGTSESAT PESSGGSSGG SRYKIGLAIG ITSVGWAVMN LDIPRIEDLG VRIFDRAENP  300
QTGESLALPR RLARSARRRL RRRKHRLERI RRLVIREGIL TKEELDKLFE EKHEIDVWQL  360
RVEALDRKLN NDELARVLLH LAKRRGFKSN RKSERSNKEN STMLKHIEEN RAILSSYRTV  420
GEMIVKDPKF ALHKRNKGEN YTNTIARDDL EREIRLIFSK QREFGNMSCT EEFENEYITI  480
WASQRPVASK DDIEKKVGFC TFEPKEKRAP KATYTFQSFI AWEHINKLRL ISPSGARGLT  540
DEERRLLYEQ AFQKNKITYH DIRTLLHLPD DTYFKGIVYD RGESRKQNEN IRFLELDAYH  600
QIRKAVDKVY GKGKSSSFLP IDFDTFGYAL TLFKDDADIH SYLRNEYEQN GKRMPNLANK  660
VYDNELIEEL LNLSFTKFGH LSLKALRSIL PYMEQGEVYS SACERAGYTF TGPKKKQKTM  720
LLPNIPPIAN PVVMRALTQA RKVVNAIIKK YGSPVSIHIE LARDLSQTFD ERRKTKKEQD  780
ENRKKNETAI RQLMEYGLTL NPTGHDIVKF KLWSEQNGRC AYSLQPIEIE RLLEPGYVEV  840
DHVIPYSRSL DDSYTNKVLV LTRENREKGN RIPAEYLGVG TERWQQFETF VLTNKQFSKK  900
KRDRLLRLHY DENEETEFKN RNLNDTRYIS RFFANFIREH LKFAESDDKQ KVYTVNGRVT  960
AHLRSRWEFN KNREESDLHH AVDAVIVACT TPSDIAKVTA FYQRREQNKE LAKKTEPHFP  1020
QPWPHFADEL RARLSKHPKE SIKALNLGNY DDQKLESLQP VFVSRMPKRS VTGAAHQETL  1080
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RRYVGIDERS GKIQTVVKTK LSEIKLDASG HFPMYGKESD PRTYEAIRQR LLEHNNDPKK  1140
AFQEPLYKPK KNGEPGPVIR TVKIIDTKNQ VIPLNDGKTV AYNSNIVRVD VFEKDGKYYC  1200
VPVYTMDIMK GILPNKAIEP NKPYSEWKEM TEDYTFRFSL YPNDLIRIEL PREKTVKTAA  1260
GEEINVKDVF VYYKTIDSAN GGLELISHDH RFSLRGVGSR TLKRFEKYQV DVLGNIYKVR  1320
GEKRVGLASS AHSKPGKTIR PLQSTRDSGG SGGSGGSTNL SDIIEKETGK QLVIQESILM  1380
LPEEVEEVIG NKPESDILVH TAYDESTDEN VMLLTSDAPE YKPWALVIQD SNGENKIKML  1440
SGGSGGSGGS TNLSDIIEKE TGKQLVIQES ILMLPEEVEE VIGNKPESDI LVHTAYDEST  1500
DENVMLLTSD APEYKPWALV IQDSNGENKI KMLSGGSPKK KRKV                   1544

SEQ ID NO: 82          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 82
tctgcagatc aytggggtgg atcccgaa                                     28

SEQ ID NO: 83          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 83
tgccgctcty ggtgaagatg accccgaa                                     28

SEQ ID NO: 84          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 84
catccygggc cggaggagcc cgtgcaaa                                     28

SEQ ID NO: 85          moltype = DNA  length = 27
FEATURE                Location/Qualifiers
misc_feature           1..27
                       note = Synthetic
source                 1..27
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 85
ayggcatctc gtggagatag caccaaa                                      27

SEQ ID NO: 86          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 86
aayggcatct cgtggagata gcaccaaa                                     28

SEQ ID NO: 87          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 87
tagggattay tggagctcct ttctcaaa                                     28

SEQ ID NO: 88          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
```

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SEQUENCE: 88
ccaytccagt gtaaaacttg tcagcgaa                                    28

SEQ ID NO: 89          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 89
cccctycccc acactatctc aatgcaaa                                    28

SEQ ID NO: 90          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 90
ctgtgtgcyg gcccatcact ttggcaaa                                    28

SEQ ID NO: 91          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 91
aattctaggc yatatacaac tacgcaaa                                    28

SEQ ID NO: 92          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 92
tcttcyccaa cttcacggct gtcccgaa                                    28

SEQ ID NO: 93          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 93
aaaggaatgc cytgttcatc aaagcgaa                                    28

SEQ ID NO: 94          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
                       note = Synthetic
source                 1..28
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 94
aggacttaya cctgcgaatt atgtcaaa                                    28

SEQ ID NO: 95          moltype = DNA  length = 26
FEATURE                Location/Qualifiers
misc_feature           1..26
                       note = Synthetic
source                 1..26
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 95
yaaaagatgt gtatgttgat tccaaa                                      26

SEQ ID NO: 96          moltype = DNA  length = 28
FEATURE                Location/Qualifiers
misc_feature           1..28
```

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```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 96
tcyaaaagat gtgtatgttg attccaaa                                    28

SEQ ID NO: 97           moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 97
cyaaaagatg tgtatgttga ttccaaa                                     27

SEQ ID NO: 98           moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 98
ccagcayggg caccagcctc agcccgaa                                    28

SEQ ID NO: 99           moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 99
gyttgtaaat attttcttct ctccaaa                                     27

SEQ ID NO: 100          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 100
ggyttgtaaa tattttcttc tctccaaa                                    28

SEQ ID NO: 101          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 101
ctgcgtccag yttcagagag gaggcaaa                                    28

SEQ ID NO: 102          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 102
aaatacayat gtactaagaa ttttcaaa                                    28

SEQ ID NO: 103          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 103
ccgacttaya gccagtaatg tcaccgaa                                    28
```

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```
SEQ ID NO: 104          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 104
ttaaaagcay ccgtggacta tatccgaa                                    28

SEQ ID NO: 105          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 105
aagygattgc agcagtgaaa tgggcaaa                                    28

SEQ ID NO: 106          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 106
agccaygggc ggggcagtgc ctttcaaa                                    28

SEQ ID NO: 107          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 107
ctggaatact tytgtagaaa accacgaa                                    28

SEQ ID NO: 108          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 108
gtgytctcca tgttcgaaca gacccaaa                                    28

SEQ ID NO: 109          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 109
gccatytgcc tctgggtaag ttgccaaa                                    28

SEQ ID NO: 110          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 110
cyggctcagc agattgcaaa ctgcaaa                                     27

SEQ ID NO: 111          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

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```
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 111
acyggctcag cagattgcaa actgcaaa                                    28

SEQ ID NO: 112          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 112
ttcgcaaacy ggctcagcag attgcaaa                                    28

SEQ ID NO: 113          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 113
cattcygcaa tccatgcata agtacaaa                                    28

SEQ ID NO: 114          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 114
ttcgatttgc ygtaccaagt gccacaaa                                    28

SEQ ID NO: 115          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 115
tatcyacaaa agtaccaaga attacaaa                                    28

SEQ ID NO: 116          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 116
taaacyggag aaaacctata caatcaaa                                    28

SEQ ID NO: 117          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 117
acttccttay tcaaacaaac ttttcaaa                                    28

SEQ ID NO: 118          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 118
gcatcytccg gatctttgag gggacaaa                                    28

SEQ ID NO: 119          moltype = DNA  length = 28
```

-continued

```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 119
ttatttgcyt ggaagtgatt ataccgaa                                    28

SEQ ID NO: 120          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 120
ggtatggtyt tctaatcgaa gggccaaa                                    28

SEQ ID NO: 121          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 121
gcacyggaag ttgggacata cagtcaaa                                    28

SEQ ID NO: 122          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 122
agaccatgca ayacagctca agatcgaa                                    28

SEQ ID NO: 123          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 123
tgggaagaca ygtaccaggg gagacaaa                                    28

SEQ ID NO: 124          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 124
tcttgaagay tgatagaaca gaaccaaa                                    28

SEQ ID NO: 125          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 125
ggaacgaygg gccttcaact tcagcgaa                                    28

SEQ ID NO: 126          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
```

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```
SEQUENCE: 126
tytggagtga taccacaggt gagcaaa                                        27

SEQ ID NO: 127          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 127
ggaacgaygg gccttcaact tcagcgaa                                       28

SEQ ID NO: 128          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 128
ctytggagtg ataccacagg tgagcaaa                                       28

SEQ ID NO: 129          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 129
gaatccaaga yggcgggatc caggcaaa                                       28

SEQ ID NO: 130          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 130
cyttgcagac tttgggaacc acacaaa                                        27

SEQ ID NO: 131          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 131
tcyttgcaga ctttgggaac cacacaaa                                       28

SEQ ID NO: 132          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 132
tttctycggc ttagaggtga ctggcaaa                                       28

SEQ ID NO: 133          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 133
tycttcggct tagaggtgac tggcaaa                                        27

SEQ ID NO: 134          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

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```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 134
ttycttcggc ttagaggtga ctggcaaa                                    28

SEQ ID NO: 135          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 135
tgggtgycca ataagaccga aggccgaa                                    28

SEQ ID NO: 136          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 136
ccaacayaat gcagatggaa gaagcaaa                                    28

SEQ ID NO: 137          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 137
taaggaccyg cagcaccggc tggacgaa                                    28

SEQ ID NO: 138          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 138
aatagagtgc yaaaagaccc aaaacaaa                                    28

SEQ ID NO: 139          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 139
tgaatccaag ygtcctctga tggtcaaa                                    28

SEQ ID NO: 140          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 140
gatatacaaa gyggaaatag acaccaaa                                    28

SEQ ID NO: 141          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 141
ggaggaaytt gtagaaggga tatacaaa                                    28
```

-continued

```
SEQ ID NO: 142          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 142
ggaggaaytt gtagaaggga tatacaaa                                    28

SEQ ID NO: 143          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 143
gatayacaaa gtggaaatag acaccaaa                                    28

SEQ ID NO: 144          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 144
cyggactttt cctccaaaga agtcaaa                                     27

SEQ ID NO: 145          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 145
ccyggacttt tcctccaaag aagtcaaa                                    28

SEQ ID NO: 146          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 146
caatcgctac cyggactttt cctccaaa                                    28

SEQ ID NO: 147          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 147
gccytgatgc tctcagcaag aaagcgaa                                    28

SEQ ID NO: 148          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 148
cgaaytcgtg gacatcatca atgccaaa                                    28

SEQ ID NO: 149          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

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```
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 149
cagtgggctg cyggtctacc agggcaaa                                    28

SEQ ID NO: 150          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 150
acactccctt yccaaggaaa agatcgaa                                    28

SEQ ID NO: 151          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 151
tggctccaac cytctgtcca tctgcaaa                                    28

SEQ ID NO: 152          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 152
atgtcaacyg gattaaggaa aaaacaaa                                    28

SEQ ID NO: 153          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 153
cctggacycc gtgcagcctg taagcaaa                                    28

SEQ ID NO: 154          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 154
gatacaytga ctatgctgaa tttgcaaa                                    28

SEQ ID NO: 155          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 155
agccagtcgt cygggattgt attccaaa                                    28

SEQ ID NO: 156          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 156
tctcyccaga cccactgctg ctctcaaa                                    28

SEQ ID NO: 157          moltype = DNA  length = 28
```

-continued

```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 157
gccayttgcc tctgggtaag ttgccaaa                                    28

SEQ ID NO: 158          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 158
ataytgccct cgacacagat ctggcaaa                                    28

SEQ ID NO: 159          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 159
cataccyggc aagcaatagc gcggcgaa                                    28

SEQ ID NO: 160          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 160
tgtatgtcya cagaaggaca gaagcaaa                                    28

SEQ ID NO: 161          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 161
agcgtcygga ttggaatcgt gagtcaaa                                    28

SEQ ID NO: 162          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 162
agataygcgt gaggttgtgt gtgtcaaa                                    28

SEQ ID NO: 163          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 163
ataccyaaag gagaaaaaga atcccaaa                                    28

SEQ ID NO: 164          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
```

-continued

```
SEQUENCE: 164
ctcaygggac ttttgaggcc actgcaaa                                    28

SEQ ID NO: 165          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 165
gaacatccyg tgccactgct tcaacaaa                                    28

SEQ ID NO: 166          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 166
aaayggccca gcctgcattt gccccaaa                                    28

SEQ ID NO: 167          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 167
ccatcyccat gaggagtggc tcatcaaa                                    28

SEQ ID NO: 168          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 168
ccagygatca tccccttttc caagcaaa                                    28

SEQ ID NO: 169          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 169
aytggaagta ctgaccaatg ttccaaa                                     27

SEQ ID NO: 170          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 170
taytggaagt actgaccaat gttccaaa                                    28

SEQ ID NO: 171          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 171
aatcayagga caagcctttg gcaccaaa                                    28

SEQ ID NO: 172          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

-continued

```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 172
taccygagtc catttagaga gttccaaa                                    28

SEQ ID NO: 173          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 173
acgtayggga gttgctgaag aacgcgaa                                    28

SEQ ID NO: 174          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 174
acctgcygcg gaccctgtcc cgagcgaa                                    28

SEQ ID NO: 175          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 175
catcycactg gcgtacgagg ctgccgaa                                    28

SEQ ID NO: 176          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 176
actggayatg gaaatagaga ggagcaaa                                    28

SEQ ID NO: 177          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 177
tgctggcacy ggcatgctgt gcctcgaa                                    28

SEQ ID NO: 178          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 178
agtcyaccta atgttggttt gattcaaa                                    28

SEQ ID NO: 179          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 179
atcaaaatay aaaaattcta cactcgaa                                    28
```

-continued

```
SEQ ID NO: 180          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 180
caggagcagg yaaatactta tcttcaaa                                    28

SEQ ID NO: 181          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 181
aaactccyca tcaccatcca cgaccgaa                                    28

SEQ ID NO: 182          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 182
cagtaaaagg yagagataat gttccaaa                                    28

SEQ ID NO: 183          moltype = DNA  length = 27
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 183
cyccgacaca tcagccctca agccaaa                                     27

SEQ ID NO: 184          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 184
ccyccgacac atcagccctc aagccaaa                                    28

SEQ ID NO: 185          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 185
aaaacggyaa gaagcagccc gaaccaaa                                    28

SEQ ID NO: 186          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 186
tccccttty atgggaggaa aacacaaa                                     28

SEQ ID NO: 187          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

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```
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 187
ccagagyatg tccatatgta tactcaaa                                    28

SEQ ID NO: 188          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 188
cagatacytg agggagagct gcagcgaa                                    28

SEQ ID NO: 189          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 189
tgtcgtagag gycacacaga gtgtcaaa                                    28

SEQ ID NO: 190          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 190
tccayaattt gtcatcctgg ctttcaaa                                    28

SEQ ID NO: 191          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 191
cgaatycgtg gacatcatca atgccaaa                                    28

SEQ ID NO: 192          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 192
tagcagaagg yctatttcat tcagcgaa                                    28

SEQ ID NO: 193          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 193
cctcactgat aycacagaag ccatcaaa                                    28

SEQ ID NO: 194          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 194
taatttcyat atgctcaccc tagacaaa                                    28

SEQ ID NO: 195          moltype = DNA  length = 27
```

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```
FEATURE                 Location/Qualifiers
misc_feature            1..27
                        note = Synthetic
source                  1..27
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 195
cyatacaaaa aggaaaaaaa aatcaaa                                        27

SEQ ID NO: 196          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 196
acyatacaaa aaggaaaaaa aaatcaaa                                       28

SEQ ID NO: 197          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 197
tcagttgcyt tctgacagcc ggtacgaa                                       28

SEQ ID NO: 198          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 198
gagcaytggg gacgataatt ttaacaaa                                       28

SEQ ID NO: 199          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 199
catcttgcgt cygatcaaag gcgccaaa                                       28

SEQ ID NO: 200          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 200
tttgtgcctg ataacgtart aacaccag                                       28

SEQ ID NO: 201          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 201
tttgtgcctg ataacgtart aacaccag                                       28

SEQ ID NO: 202          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
```

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```
SEQUENCE: 202
tttgatatat tgttctttgr ccctgcca                                    28

SEQ ID NO: 203          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 203
ttcggctgcg acctctgtgg gargggtt                                    28

SEQ ID NO: 204          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 204
tttgacaacc ccgtctrtca gaagacca                                    28

SEQ ID NO: 205          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 205
tttgctgttg cctttggcrt caacgttc                                    28

SEQ ID NO: 206          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 206
tttggaagtt gttggcrggt acagtcca                                    28

SEQ ID NO: 207          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 207
tttgttttca aggtgttctt tgcagrag                                    28

SEQ ID NO: 208          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 208
tttgatttgc cagtttagrt gcaaaatc                                    28

SEQ ID NO: 209          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 209
tttgggcaaa atccctcrga cagtaaga                                    28

SEQ ID NO: 210          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

-continued

```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 210
tttgccttta ttgcaagggr agtcctta                                    28

SEQ ID NO: 211          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 211
tttgagaaaa gaagtactcg arcaagat                                    28

SEQ ID NO: 212          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 212
tttgcagggt ttggtttcta acctgrct                                    28

SEQ ID NO: 213          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 213
tttggtttct aacctgrctc tcagttcc                                    28

SEQ ID NO: 214          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 214
tttggaggtt acgtgtgtac arcctcac                                    28

SEQ ID NO: 215          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 215
tttgtgcacg actgcgtcaa tatcrcaa                                    28

SEQ ID NO: 216          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 216
tttgtgcacg actgcgtcaa tatcrcaa                                    28

SEQ ID NO: 217          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 217
tttgtgttta tgttatttgc artgtttt                                    28
```

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```
SEQ ID NO: 218          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 218
tttgatgtct ttcctgatgg atgggrca                                    28

SEQ ID NO: 219          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 219
tttgtattac gaattatacr gaagacaa                                    28

SEQ ID NO: 220          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 220
tttgttttgt tttctgtrgg tttcagat                                    28

SEQ ID NO: 221          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 221
tttgggaatt cctggcaggr gtggtgta                                    28

SEQ ID NO: 222          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 222
tttgaacgtt ttcctttcaa tarctgct                                    28

SEQ ID NO: 223          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 223
tttgccggag gacagagact gacrtgga                                    28

SEQ ID NO: 224          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 224
tttgggaaat gcaacaatcc aratggcc                                    28

SEQ ID NO: 225          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

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```
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 225
tttgctgaca aagaagaagg tggagrtg                                    28

SEQ ID NO: 226          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 226
tttgtccaaa gcaggtattg rgacaact                                    28

SEQ ID NO: 227          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 227
ttcgcgcgtc ttctcctccr ggtgtgca                                    28

SEQ ID NO: 228          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 228
tttggaggaa cccatgggaa tgrgctaa                                    28

SEQ ID NO: 229          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 229
tttgatgatg atgacargat cgttgggg                                    28

SEQ ID NO: 230          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 230
tttgcccctt gacaggtatg ggccrgca                                    28

SEQ ID NO: 231          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 231
tttggggtct ctgttcccgg trgagtgg                                    28

SEQ ID NO: 232          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 232
tttgagacct atgcagrtgc cctgtggt                                    28

SEQ ID NO: 233          moltype = DNA  length = 28
```

-continued

```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 233
ttcgggacgt ggcgggggra ttttacaa                                    28

SEQ ID NO: 234          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 234
tttgaaactg atgaacgctr ccgacatt                                    28

SEQ ID NO: 235          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 235
tttgggctcc aggtgcggcg gcrgcaca                                    28

SEQ ID NO: 236          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 236
tttggggcaa cgcgggaatr tgacagat                                    28

SEQ ID NO: 237          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 237
tttgcttctt tctgccagtc arcaatgt                                    28

SEQ ID NO: 238          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 238
tttgcctctt tacagccatc ccttcrtc                                    28

SEQ ID NO: 239          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 239
tttgcctctt tacagccatc ccttcrtc                                    28

SEQ ID NO: 240          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
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SEQUENCE: 240
tttggagcta cctctagtat craaagtg                                    28

SEQ ID NO: 241          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 241
tttgaacgaa ctcttaaagg acrtacag                                    28

SEQ ID NO: 242          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 242
ttcgtggcgc aggcgcctra caactacc                                    28

SEQ ID NO: 243          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 243
ttcgtggcgc aggcgcctra caactacc                                    28

SEQ ID NO: 244          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 244
tttggtccac ctgggartgg gaagacaa                                    28

SEQ ID NO: 245          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 245
tttggctttc ctgtgcacta cacagrcg                                    28

SEQ ID NO: 246          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 246
tttgtcttct ctctggggca grggctgg                                    28

SEQ ID NO: 247          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 247
ttcggaaggt ggtgagcrtc gagcaggg                                    28

SEQ ID NO: 248          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

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                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 248
tttgataagc tgaatgtaag grcaacac                                    28

SEQ ID NO: 249          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 249
ttcgggtgga gaagacaggg cacrgtgg                                    28

SEQ ID NO: 250          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 250
ttcgtatttt ggatgtcart gacaatat                                    28

SEQ ID NO: 251          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 251
tttgttcgaa agaatggtgt cratgaag                                    28

SEQ ID NO: 252          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 252
ttcgaaagaa tggtgtcrat gaagccaa                                    28

SEQ ID NO: 253          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 253
tttggtcttg ccagagacat gtrtgata                                    28

SEQ ID NO: 254          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 254
tttgagacta aatcacarca aactgtca                                    28

SEQ ID NO: 255          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 255
tttggacaaa aaggctggcc caaargat                                    28
```

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SEQ ID NO: 256          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 256
tttggaccct gaggtcagac carcttca                                    28

SEQ ID NO: 257          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 257
tttgaagttg cttgggaagt gaccarta                                    28

SEQ ID NO: 258          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 258
tttgccactt tggctgagtt ggtccrgt                                    28

SEQ ID NO: 259          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 259
tttggctgag ttggtccrgt attacatg                                    28

SEQ ID NO: 260          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 260
tttgttatgc tttcaaatra gaaagatt                                    28

SEQ ID NO: 261          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 261
tttgtttgtt ccggtcggaa accragga                                    28

SEQ ID NO: 262          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 262
tttgttccgg tcggaaaccr aggacctt                                    28

SEQ ID NO: 263          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

-continued

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                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 263
tttgaatcct tcaaacgtgc tgrcatct                                    28

SEQ ID NO: 264          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 264
tttggatctg ttttaggcct rtggaggt                                    28

SEQ ID NO: 265          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 265
tttgctgtgc ccaacccgaa gcrggtaa                                    28

SEQ ID NO: 266          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 266
ttcgttaata tggagagagr tctagctt                                    28

SEQ ID NO: 267          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 267
ttcgtgaact tcggcgtcgg ggrcttga                                    28

SEQ ID NO: 268          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 268
tttgaaccca aagacatcrc tgctgaag                                    28

SEQ ID NO: 269          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 269
tttgcttttg taggttgccr tgggtcaa                                    28

SEQ ID NO: 270          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 270
tttggggttt tattttcrgg ttactgga                                    28

SEQ ID NO: 271          moltype = DNA  length = 28
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```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 271
tttgggtgct ccggggrtga caccagta                                    28

SEQ ID NO: 272          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 272
tttgtcgaaa gctgcttttc crgggttt                                    28

SEQ ID NO: 273          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 273
ttcgtataat attctctrcg ttaggagt                                    28

SEQ ID NO: 274          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 274
tttgttaaga tggcagrgcc cggtaatc                                    28

SEQ ID NO: 275          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 275
tttgttaaga tggcagagcc cggtartc                                    28

SEQ ID NO: 276          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 276
tttgttaaga tggcagagcc cggtratc                                    28

SEQ ID NO: 277          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 277
tttgcggaga ggtcagtaac traattgg                                    28

SEQ ID NO: 278          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
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SEQUENCE: 278
tttgtctttc tctttttaa araattca                                    28

SEQ ID NO: 279          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 279
tttgtctttc tctttttaa araattca                                    28

SEQ ID NO: 280          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 280
tttgcaggag gcattgraat tttcagca                                   28

SEQ ID NO: 281          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 281
tttgtccagg cttgagtcac catgrgta                                   28

SEQ ID NO: 282          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 282
tttgtactga gccacatgac arggtaat                                   28

SEQ ID NO: 283          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 283
tttgacttcc tcgggaaggr ctccatca                                   28

SEQ ID NO: 284          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 284
tttggaagtt gttggcaggt rcagtcca                                   28

SEQ ID NO: 285          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 285
tttgctttct aaacactttt cartgttt                                   28

SEQ ID NO: 286          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

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                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 286
tttggacttt gctatctttc rggttttg                                    28

SEQ ID NO: 287          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 287
tttgcaaact cgtaatttct ttcrggag                                    28

SEQ ID NO: 288          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 288
tttgtttgcc tgaatgacar cattgacc                                    28

SEQ ID NO: 289          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 289
tttggctgac ctgcctagaa atattrtg                                    28

SEQ ID NO: 290          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 290
ttcggaccaa tcctgagrct tgttacca                                    28

SEQ ID NO: 291          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 291
tttgtcttct cttcttrggg ccctcctg                                    28

SEQ ID NO: 292          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 292
ttcgtcgctt atgcatrctc aggatgag                                    28

SEQ ID NO: 293          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 293
ttcgagggca cgttccrtgt gaacacgg                                    28
```

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```
SEQ ID NO: 294          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 294
tttgacaacg acttcccagc tctgcrgc                                    28

SEQ ID NO: 295          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 295
ttcgagcttc tcagtatatt tagtcrta                                    28

SEQ ID NO: 296          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 296
tttgttttcc cctttaaaca rttaagat                                    28

SEQ ID NO: 297          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 297
tttgcagaaa gggggccctg rggcagaa                                    28

SEQ ID NO: 298          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 298
tttgtgctac agaaactrct atgctgac                                    28

SEQ ID NO: 299          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 299
tttgtgctac agaaactact rtgctgac                                    28

SEQ ID NO: 300          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 300
ttcgactcat taaattrtga taatcata                                    28

SEQ ID NO: 301          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

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```
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 301
tttgcaattc aatatgaaaa tcrcctcg                                    28

SEQ ID NO: 302        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 302
tttgcaattc aatatgaaaa tcrcctcg                                    28

SEQ ID NO: 303        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 303
tttgactgct ttttcacrat tgatctct                                    28

SEQ ID NO: 304        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 304
tttgccctac aacagtacar gttatcca                                    28

SEQ ID NO: 305        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 305
tttggattca gtagcagagr atacgtct                                    28

SEQ ID NO: 306        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 306
tttgttttcc ctcaaaggtt carcatac                                    28

SEQ ID NO: 307        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 307
tttggatcaa cctcacaatc argtagtt                                    28

SEQ ID NO: 308        moltype = DNA  length = 28
FEATURE               Location/Qualifiers
misc_feature          1..28
                      note = Synthetic
source                1..28
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 308
tttgccctca gcatctccct tcrtgtct                                    28

SEQ ID NO: 309        moltype = DNA  length = 28
```

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```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 309
tttgggatag ctatacaaca aatraggt                                    28

SEQ ID NO: 310          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 310
ttcgtggctc cagagcract gtggccct                                    28

SEQ ID NO: 311          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 311
tttgtgatgg aatatgttar tgggggcg                                    28

SEQ ID NO: 312          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 312
tttgttttgt tgtgtttttc trgaggat                                    28

SEQ ID NO: 313          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 313
tttgttgtgt ttttctrgag gatttcaa                                    28

SEQ ID NO: 314          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 314
tttggttttt gcccttccag tgtrtact                                    28

SEQ ID NO: 315          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 315
tttgcccttc cagtgtrtac tctgaaag                                    28

SEQ ID NO: 316          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
```

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SEQUENCE: 316
tttgtttgtt tgttttgtca ctrgcacg                                    28

SEQ ID NO: 317          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 317
tttgtttgtt ttgtcactrg cacgggga                                    28

SEQ ID NO: 318          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 318
tttgtgtttg tgctgcrggt gggcaagt                                    28

SEQ ID NO: 319          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 319
ttcgctgcaa accagaccrt ggatactg                                    28

SEQ ID NO: 320          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 320
tttgctttgt tttattttrg tcctgttg                                    28

SEQ ID NO: 321          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 321
tttgtttgct tttttcctgt acrggaaa                                    28

SEQ ID NO: 322          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 322
tttgcttttt tcctgtacrg gaaactgg                                    28

SEQ ID NO: 323          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 323
tttgatcaat tagatcttgt cacatrtg                                    28

SEQ ID NO: 324          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

-continued

```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 324
tttgttctca ctgttttctt tctttrgg                                    28

SEQ ID NO: 325          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 325
tttgttttgt ttttccrgat tcgctttg                                    28

SEQ ID NO: 326          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 326
tttgaggcct gtggttgara tggaaata                                    28

SEQ ID NO: 327          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 327
tttgtatctt ttcatctrgc taaaagac                                    28

SEQ ID NO: 328          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 328
tttgtgttga taagctctac ggtgtraa                                    28

SEQ ID NO: 329          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 329
tttgaggatg atgtcartga gttcacct                                    28

SEQ ID NO: 330          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 330
tttgctggct ttcttcacrg aagtaaaa                                    28

SEQ ID NO: 331          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 331
tttgtgaaat tccaccrtgg cgtaccgt                                    28
```

-continued

```
SEQ ID NO: 332          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 332
tttgatcatt gtctctggtg rgcgatat                                    28

SEQ ID NO: 333          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 333
tttggaattt atccagtgat caggtrtt                                    28

SEQ ID NO: 334          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 334
tttgttccct ttcttacrga tttaatgt                                    28

SEQ ID NO: 335          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 335
tttgtatata tattttttat ctttcrgc                                    28

SEQ ID NO: 336          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 336
ttcgatgttc ctcttaaagt ggrtctca                                    28

SEQ ID NO: 337          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 337
ttcggactct caagcaggaa racattgt                                    28

SEQ ID NO: 338          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 338
tttgtctctt ctcttcttat rggacctg                                    28

SEQ ID NO: 339          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
```

-continued

```
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 339
tttgattata attttggatc rtctgtat                                    28

SEQ ID NO: 340          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 340
tttgatctta gtgtttatta cagraaat                                    28

SEQ ID NO: 341          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 341
tttgtgcaag tttgcaaacr tgttcacc                                    28

SEQ ID NO: 342          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 342
tttgtgaaac tgctttttga aacagrga                                    28

SEQ ID NO: 343          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 343
tttgttttcc aagacccacc ratgacat                                    28

SEQ ID NO: 344          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 344
ttcgtccttg accgcagggt cctggrta                                    28

SEQ ID NO: 345          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 345
ttcgggaatg tgacgactat tttccrac                                    28

SEQ ID NO: 346          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 346
ttcgggaatg tgacgactat tttccrac                                    28

SEQ ID NO: 347          moltype = DNA  length = 28
```

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```
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 347
tttgtgctgg agcaggrgga gtacaaga                                    28

SEQ ID NO: 348          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 348
tttgacttca cggtaactgg grgaggga                                    28

SEQ ID NO: 349          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 349
tttgttttca ttcacccrgg aatgcatt                                    28

SEQ ID NO: 350          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 350
tttggcaata atgaaaaggt aarttgat                                    28

SEQ ID NO: 351          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 351
tttgtggaga catccctgrg acggccac                                    28

SEQ ID NO: 352          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 352
tttgctgtaa atcaaatcat gggcrgcc                                    28

SEQ ID NO: 353          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 353
tttgcatgta tttcctrggg atgtcctt                                    28

SEQ ID NO: 354          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
```

-continued

```
SEQUENCE: 354
tttgtaaaac ctctagraat gtctcata                                    28

SEQ ID NO: 355          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 355
tttgatttct tttttttca ttggcrga                                     28

SEQ ID NO: 356          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 356
tttggtccac ctgggaatgg gaagrcaa                                    28

SEQ ID NO: 357          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 357
tttgtatacg tgtaatgcar cattcaga                                    28

SEQ ID NO: 358          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 358
tttgaattct atttcactrg ttccttcc                                    28

SEQ ID NO: 359          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 359
tttgaatcct tgctctgcga tggrctat                                    28

SEQ ID NO: 360          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 360
tttgccattg ctaaggaatt gctaraaa                                    28

SEQ ID NO: 361          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 361
tttgcctctc tcctcacrgc ccccagtc                                    28

SEQ ID NO: 362          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
```

-continued

```
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 362
tttgacaaac tgtgtgtttr tgacaatg                                    28

SEQ ID NO: 363          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 363
tttgcttttt tctccctccc cccargat                                    28

SEQ ID NO: 364          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 364
ttcggcaacg tctctcccaa crccaact                                    28

SEQ ID NO: 365          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 365
ttcggcaacg tctctcccaa crccaact                                    28

SEQ ID NO: 366          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 366
tttgcactta gggggccagg acrtcttc                                    28

SEQ ID NO: 367          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 367
ttcgaggggc tccgcaagca ctrcctgc                                    28

SEQ ID NO: 368          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 368
tttgctgttc ttcacagctt taggrtat                                    28

SEQ ID NO: 369          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 369
tttgatgctt gtttaattag tgragaat                                    28
```

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```
SEQ ID NO: 370          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 370
ttcgctgtca tattcctggc trgctaca                                    28

SEQ ID NO: 371          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 371
tttgtcttcc ttgacgatat tctrcttt                                    28

SEQ ID NO: 372          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 372
tttgtcttcc ttgacgatat tctrcttt                                    28

SEQ ID NO: 373          moltype = DNA  length = 28
FEATURE                 Location/Qualifiers
misc_feature            1..28
                        note = Synthetic
source                  1..28
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 373
tttgcaatgg cctccagrta attcttcc                                    28

SEQ ID NO: 374          moltype = AA  length = 18
FEATURE                 Location/Qualifiers
REGION                  1..18
                        note = Synthetic
source                  1..18
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 374
KRTADGSEFE SPKKKRKV                                               18

SEQ ID NO: 375          moltype = AA  length = 17
FEATURE                 Location/Qualifiers
REGION                  1..17
                        note = Synthetic
source                  1..17
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 375
KRTADGSEFE PKKKRKV                                                17

SEQ ID NO: 376          moltype = DNA  length = 125
FEATURE                 Location/Qualifiers
misc_feature            1..125
                        note = Synthetic
misc_difference         1..8
                        note = n is a, c, g, or t
source                  1..125
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 376
nnnnnnnngt ttttgtactc tcaagattta gaaataaatc ttgcagaagc tacaaagata  60
aggcttcatg ccgaaatcaa caccctgtca ttttatggca gggtgttttc gttatttaat  120
ttttt                                                             125

SEQ ID NO: 377          moltype = DNA  length = 121
```

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```
FEATURE                 Location/Qualifiers
misc_feature            1..121
                        note = Synthetic
misc_difference         1..18
                        note = n is a, c, g, or t
source                  1..121
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 377
nnnnnnnnnn nnnnnnnngt ttttgtactc tcagaaatgc agaagctaca aagataaggc  60
ttcatgccga aatcaacacc ctgtcatttt atggcagggt gttttcgtta tttaattttt  120
t                                                                  121

SEQ ID NO: 378          moltype = DNA  length = 109
FEATURE                 Location/Qualifiers
misc_feature            1..109
                        note = Synthetic
misc_difference         1..20
                        note = n is a, c, g, or t
source                  1..109
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 378
nnnnnnnnnn nnnnnnnnnn gtttttgtac tctcagaaat gcagaagcta caaagataag  60
gcttcatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgtttttt              109

SEQ ID NO: 379          moltype = DNA  length = 102
FEATURE                 Location/Qualifiers
misc_feature            1..102
                        note = Synthetic
misc_difference         1..20
                        note = n is a, c, g, or t
source                  1..102
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 379
nnnnnnnnnn nnnnnnnnnn gttttagagc tagaaatagc aagttaaaat aaggctagtc  60
cgttatcaac ttgaaaaagt ggcaccgagt cggtgctttt tt                     102

SEQ ID NO: 380          moltype = DNA  length = 88
FEATURE                 Location/Qualifiers
misc_feature            1..88
                        note = Synthetic
misc_difference         1..20
                        note = n is a, c, g, or t
source                  1..88
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 380
nnnnnnnnnn nnnnnnnnnn gttttagagc tagaaatagc aagttaaaat aaggctagtc  60
cgttatcaac ttgaaaaagt gtttttt                                      88

SEQ ID NO: 381          moltype = DNA  length = 76
FEATURE                 Location/Qualifiers
misc_feature            1..76
                        note = Synthetic
misc_difference         1..20
                        note = n is a, c, g, or t
source                  1..76
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 381
nnnnnnnnnn nnnnnnnnnn gttttagagc tagaaatagc aagttaaaat aaggctagtc  60
cgttatcatt tttttt                                                  76

SEQ ID NO: 382          moltype = RNA  length = 81
FEATURE                 Location/Qualifiers
misc_feature            1..81
                        note = Synthetic
source                  1..81
                        mol_type = other RNA
                        organism = synthetic construct
SEQUENCE: 382
gttttagagc tagaaatagc aagttaaaat aaggctagtc cgttatcaac ttgaaaaagt  60
ggcaccgagt cggtgctttt t                                            81
```

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```
SEQ ID NO: 383          moltype = AA  length = 36
FEATURE                 Location/Qualifiers
REGION                  1..36
                        note = Synthetic
VARIANT                 2
                        note = X can be any naturally occurring amino acid
VARIANT                 4..29
                        note = X can be any naturally occurring amino acid
REGION                  26..29
                        note = may or may not be present
VARIANT                 32..35
                        note = X can be any naturally occurring amino acid
REGION                  34..35
                        note = may or may not be present
source                  1..36
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 383
HXEXXXXXXX XXXXXXXXXX XXXXXXXXXP CXXXXC                            36

SEQ ID NO: 384          moltype = AA  length = 20
FEATURE                 Location/Qualifiers
REGION                  1..20
                        note = Synthetic
source                  1..20
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 384
NNNNAAASSW WSSTTTNNNN                                              20

SEQ ID NO: 385          moltype = AA  length = 343
FEATURE                 Location/Qualifiers
REGION                  1..343
                        note = Synthetic
source                  1..343
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 385
MSNLLTVHQN LPALPVDATS DEVRKNLMDM FRDRQAFSEH TWKMLLSVCR SWAAWCKLNN  60
RKWFPAEPED VRDYLLYLQA RGLAVKTIQQ HLGQLNMLHR RSGLPRPSDS NAVSLVMRRI  120
RKENVDAGER AKQALAFERT DFDQVRSLME NSDRCQDIRN LAFLGIAYNT LLRIAEIARI  180
RVKDISRTDG GRMLIHIGRT KTLVSTAGVE KALSLGVTKL VERWISVSGV ADDPNNYLFC  240
RVRKNGVAAP SATSQLSTRA LEGIFEATHR LIYGAKDDSG QRYLAWSGHS ARVGAARDMA  300
RAGVSIPEIM QAGGWTNVNI VMNYIRNLDS ETGAMVRLLE DGD                    343

SEQ ID NO: 386          moltype = AA  length = 423
FEATURE                 Location/Qualifiers
REGION                  1..423
                        note = Synthetic
source                  1..423
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 386
MPQFGILCKT PPKVLVRQFV ERFERPSGEK IALCAAELTY LCWMITHNGT AIKRATFMSY  60
NTIISNSLSF DIVNKSLQFK YKTQKATILE ASLKKLIPAW EFTIIPYYGQ KHQSDITDIV  120
SSLQLQFESS EEADKGNSHS KKMLKALLSE GESIWEITEK ILNSFEYTSR FTKTKTLYQF  180
LFLATFINCG RFSDIKNVDP KSFKLVQNKY LGVIIQCLVT ETKTSVSRHI YFFSARGRID  240
PLVYLDEFLR NSEPVLKRVN RTGNSSSNKQ EYQLLKDNLV RSYNKALKKN APYSIFAIKN  300
GPKSHIGRHL MTSFLSMKGL TELTNVVGNW SDKRASAVAR TTYTHQITAI PDHYFALVSR  360
YYAYDPISKE MIALKDETNP IEEWQHIEQL KGSAEGSIRY PAWNGIISQE VLDYLSSYIN  420
RRI                                                                423

SEQ ID NO: 387          moltype = AA  length = 144
FEATURE                 Location/Qualifiers
REGION                  1..144
                        note = Synthetic
source                  1..144
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 387
MRLFGYARVS TSQQSLDIQV RALKDAGVKA NRIFTDKASG SSSDRKGLDL LRMKVEEGDV  60
ILVKKLDRLG RDTADMIQLI KEFDAQGVSI RFIDDGISTD GEMGKMVVTI LSAVAQAERQ  120
RILERTNEGR QEAMAKGVVF GRKR                                         144

SEQ ID NO: 388          moltype = AA  length = 144
FEATURE                 Location/Qualifiers
```

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```
REGION                  1..144
                        note = Synthetic
source                  1..144
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 388
MRLFGYARVS TSQQSLDIQV RALKDAGVKA NRIFTDKASG SSSDRKGLDL LRMKVEEGDV  60
ILVKKLDRLG RDTADMIQLI KEFDAQGVSI RFIDDGISTD GEMGKMVVTI LSAVAQAERQ  120
RILQRTNEGR QEAMAKGVVF GRKR                                         144

SEQ ID NO: 389          moltype = AA  length = 144
FEATURE                 Location/Qualifiers
REGION                  1..144
                        note = Synthetic
source                  1..144
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 389
MRLFGYARVS TSQQSLDIQV RALKDAGVKA NRIFTDKASG SSSDRKGLDL LRMKVEEGDV  60
ILVKKLDRLG RDTADMIQLI KEFDAQGVSI RFIDDGISTD GYMGKMVVTI LSAVAQAERQ  120
RILQRTNEGR QEAMAKGVVF GRKR                                         144

SEQ ID NO: 390          moltype = AA  length = 147
FEATURE                 Location/Qualifiers
REGION                  1..147
                        note = Synthetic
source                  1..147
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 390
MAKIGYARVS SKEQNLDRQL QALQGVSKVF SDKLSGQSVE RPQLQAMLNY IREGDIVVVT  60
ELDRLGRNNK ELTELMNAIQ QKGATLEVLD LPSMNGIEDE NLRRLINNLV IELYKYQAES  120
ERKRIKERQA QGIEIAKSKG KFKGRQH                                      147

SEQ ID NO: 391          moltype = AA  length = 147
FEATURE                 Location/Qualifiers
REGION                  1..147
                        note = Synthetic
source                  1..147
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 391
MAKIGYARVS SKEQNLDRQL QALQGVSKVF SDKLSGQSVE RPQLQAMLNY IREGDIVVVT  60
ELDRLGRNNK ELTELMNAIQ QKGATLEVLD LPSMDGIEDE NLRRLINNLV IELYKYQAES  120
ERKRIKERQA QGIEIAKSKG KFKGRQH                                      147

SEQ ID NO: 392          moltype = AA  length = 150
FEATURE                 Location/Qualifiers
REGION                  1..150
                        note = Synthetic
source                  1..150
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 392
MIIGYARVSS LDQNLERQLE NLKTFGAEKI FTEKQSGKSI ENRPILQKAL NFVRMGDRFI  60
VESIDRLGRN YNEVIHTVNY LKDKEVQLMI TSLPMMNEVI GNPLLDKFMK DLIIQILAMV  120
SEQERNESKR RQAQGIQVAK EKGVYKGRPL                                   150

SEQ ID NO: 393          moltype = AA  length = 150
FEATURE                 Location/Qualifiers
REGION                  1..150
                        note = Synthetic
source                  1..150
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 393
MIIGYARVSS LDQNLERQLE NLKTFGAEKI FTEKQSGKSI ENRPILQKAL NFVRMGDRFI  60
VESIDRLGRN YNEVIHTVNY LKDKEVRLMI TSLPMMNEVI GNPLLDKFMK DLIIRILAMV  120
SEQERNESKR RQAQGIQVAK EKGVYKGRPL                                   150

SEQ ID NO: 394          moltype = AA  length = 144
FEATURE                 Location/Qualifiers
REGION                  1..144
                        note = Synthetic
source                  1..144
                        mol_type = protein
```

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```
                        organism = synthetic construct
SEQUENCE: 394
MRLFGYARVS TSQQSLDLQV RALKDAGVKA NRIFTDKASG SSTDREGLDL LRMKVKEGDV  60
ILVKKLDRLG RDTADMLQLI KEFDAQGVAV RFIDDGISTD GDMGQMVVTI LSAVAQAERR  120
RILERTNEGR QEAKLKGIKF GRRR                                         144

SEQ ID NO: 395          moltype = AA  length = 144
FEATURE                 Location/Qualifiers
REGION                  1..144
                        note = Synthetic
source                  1..144
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 395
MRLFGYARVS TSQQSLDLQV RALKDAGVKA NRIFTDKASG SSTDREGLDL LRMKVKEGDV  60
ILVKKLDRLS RDTADMLQLI KEFDAQGVAV RFIDDGISTD GYMGQMVVTI LSAVAQAERR  120
RILQRTNEGR QEAKLKGIKF GRRR                                         144

SEQ ID NO: 396          moltype = AA  length = 142
FEATURE                 Location/Qualifiers
REGION                  1..142
                        note = Synthetic
source                  1..142
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 396
MATIGYIRVS TIDQNIDLQR NALTSANCDR IFEDRISGKI ANRPGLKRAL KYVNKGDTLV  60
VWKLDRLGRS VKNLVALISE LHERGAHFHS LTDSIDTSSA MGRFFFHVMS ALAEMERELI  120
VERTLAGLAA ARAQGRLGGR PV                                           142

SEQ ID NO: 397          moltype = AA  length = 142
FEATURE                 Location/Qualifiers
REGION                  1..142
                        note = Synthetic
source                  1..142
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 397
MATIGYIRVS TIDQNIDLQR NALTSANCDR IFEDRISGKI ANRPGLKRAL KYVNKGDTLV  60
VWKLDRLGRS VKNLVALISE LHERGAHFHS LTDSIDTSSA MGRFFFYVMS ALAEMERELI  120
VERTLAGLAA ARAQGRLGGR PV                                           142

SEQ ID NO: 398          moltype = AA  length = 608
FEATURE                 Location/Qualifiers
REGION                  1..608
                        note = Synthetic
source                  1..608
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 398
MDTYAGAYDR QSRERENSSA ASPATQRSAN EDKAADLQRE VERDGGRFRF VGHFSEAPGT  60
SAFGTAERPE FERILNECRA GRLNMIIVYD VSRFSRLKVM DAIPIVSELL ALGVTIVSTQ  120
EGVFRQGNVM DLIHLIMRLD ASHKESSLKS AKILDTKNLQ RELGGYVGGK APYGFELVSE  180
TKEITRNGRM VNVVINKLAH STTPLTGPFE FEPDVIRWWW REIKTHKHLP FKPGSQAAIH  240
PGSITGLCKR MDADAVPTRG ETIGKKTASS AWDPATVMRI LRDPRIAGFA AEVIYKKKPD  300
GTPTTKIEGY RIQRDPITLR PVELDCGPII EPAEWYELQA WLDGRGRGKG LSRGQAILSA  360
MDKLYCECGA VMTSKRGEES IKDSYRCRRR KVVDPSAPGQ HEGTCNVSMA ALDKFVAERI  420
FNKIRHAEGD EETLALLWEA ARRFGKLTEA PEKSGERANL VAERADALNA LEELYEDRAA  480
GAYDGPVGRK HFRKQQAALT LRQQGAEERL AELEAAEAPK LPLDQWFPED ADADPTGPKS  540
WWGRASVDDK RVFVGLFVDK IVVTKSTTGR GQGTPIEKRA SITWAKPPTD DDEDDAQDGT  600
EDVAATGA                                                           608

SEQ ID NO: 399          moltype = DNA  length = 34
FEATURE                 Location/Qualifiers
misc_feature            1..34
                        note = Synthetic
source                  1..34
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 399
ataacttcgt atagcataca ttatacgaag ttat                              34

SEQ ID NO: 400          moltype = DNA  length = 34
FEATURE                 Location/Qualifiers
misc_feature            1..34
                        note = Synthetic
```

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source                  1..34
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 400
gaagttccta ttctctagaa agtataggaa cttc                                34

SEQ ID NO: 401          moltype = AA  length = 173
FEATURE                 Location/Qualifiers
REGION                  1..173
                        note = Synthetic
source                  1..173
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 401
AKPAKRIKSA AAAYVPQNRD AVITDIKRIG DLQREASRLE TEMNDAIAEI TEKFAARIAP  60
IKTDIETLSK GVQGWCEANR DELTNGGKVK TANLVTGDVS WRVRPPSVSI RGMDAVMETL  120
ERLGLQRFIR TKQEINKEAI LLEPKAVAGV AGITVKSGIE DFSIIPFEQE AGI         173

SEQ ID NO: 402          moltype = AA  length = 174
FEATURE                 Location/Qualifiers
REGION                  1..174
                        note = Synthetic
source                  1..174
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 402
MAKPAKRIKS AAAAYVPQNR DAVITDIKRI GDLQREASRL ETEMNDAIAE ITEKFAARIA  60
PIKTDIETLS KGVQGWCEAN RDELTNGGKV KTANLVTGDV SWRVRPPSVS IRGMDAVMET  120
LERLGLQRFI RTKQEINKEA ILLEPKAVAG VAGITVKSGI EDFSIIPFEQ EAGI        174

SEQ ID NO: 403          moltype = AA  length = 174
FEATURE                 Location/Qualifiers
source                  1..174
                        mol_type = protein
                        organism = Escherichia coli
SEQUENCE: 403
MAKPAKRIKS AAAAYVPQNR DAVITDIKRI GDLQREASRL ETEMNDAIAE ITEKFAARIA  60
PIKTDIETLS KGVQGWCEAN RDELTNGGKV KTANLVTGDV SWRVRPPSVS IRGMDAVMET  120
LERLGLQRFV RTKQEINKEA ILLEPKAVAG VAGITVKSGI EDFSIIPFEQ EAGI        174

SEQ ID NO: 404          moltype = AA  length = 174
FEATURE                 Location/Qualifiers
source                  1..174
                        mol_type = protein
                        organism = Escherichia coli
SEQUENCE: 404
MAKPAKRIKS AAAAYVPQNR DAVITDIKRI GDLQREASRL ETEMNDAIAE ITEKFAARIA  60
PLKTDIETLS KGVQGWCEAN RDELTNGGKV KTANLVTGDV SWRVRPPSVS IRGMDAVMET  120
LERLGLQRFI RTKQEINKEA ILLEPKAVAG VAGITVKSGI EDFSIIPFEQ EAGI        174

SEQ ID NO: 405          moltype = AA  length = 174
FEATURE                 Location/Qualifiers
source                  1..174
                        mol_type = protein
                        organism = Escherichia coli
SEQUENCE: 405
MAKPAKRIKS AAAAYVPQNR DAVITDIKRI GDLQREASRL ETEMNDAIAE ITEKFAARIA  60
PIKTDIETLS KGVQGWCEAN RDELTNGGKV KTANLITGDV SWRVRPPSVS IRGMDAVMET  120
LERLGLQRFI RTKQEINKEA ILLEPKAVAG VAGITVKSGI EDFSIIPFEQ EAGI        174

SEQ ID NO: 406          moltype = DNA  length = 31
FEATURE                 Location/Qualifiers
misc_feature            1..31
                        note = Synthetic
source                  1..31
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 406
tctctgcgta ctgattggaa catccgcgaa a                                   31

SEQ ID NO: 407          moltype = DNA  length = 31
FEATURE                 Location/Qualifiers
misc_feature            1..31
                        note = Synthetic
source                  1..31
                        mol_type = other DNA
```

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                        organism = synthetic construct
SEQUENCE: 407
gctgcagaag ggattccatg aggtgcgcga a                                  31

SEQ ID NO: 408          moltype = DNA  length = 30
FEATURE                 Location/Qualifiers
misc_feature            1..30
                        note = Synthetic
source                  1..30
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 408
gcggggcggg ccagcctgaa tagctgcaaa                                    30

SEQ ID NO: 409          moltype = DNA  length = 31
FEATURE                 Location/Qualifiers
misc_feature            1..31
                        note = Synthetic
source                  1..31
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 409
gctggccctg taaaggaaac tggaacacaa a                                  31

SEQ ID NO: 410          moltype = DNA  length = 31
FEATURE                 Location/Qualifiers
misc_feature            1..31
                        note = Synthetic
source                  1..31
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 410
gtggttccag aaccggagga caaagtacaa a                                  31

SEQ ID NO: 411          moltype = DNA  length = 30
FEATURE                 Location/Qualifiers
misc_feature            1..30
                        note = Synthetic
source                  1..30
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 411
tcacatcaac cggtggcgca ttgccacgaa                                    30

SEQ ID NO: 412          moltype = DNA  length = 30
FEATURE                 Location/Qualifiers
misc_feature            1..30
                        note = Synthetic
source                  1..30
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 412
gaaggtgtgg ttccagaacc ggaggacaaa                                    30

SEQ ID NO: 413          moltype = AA  length = 167
FEATURE                 Location/Qualifiers
source                  1..167
                        mol_type = protein
                        organism = Escherichia coli
SEQUENCE: 413
MSEVEFSHEY WMRHALTLAK RAWDEREVPV GAVLVHNNRV IGEGWNRPIG RHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTLEPCVMC AGAMIHSRIG RVVFGARDAK TGAAGSLMDV  120
LHHPGMNHRV EITEGILADE CAALLSDFFR MRRQEIKAQK KAQSSTD                167

SEQ ID NO: 414          moltype = AA  length = 178
FEATURE                 Location/Qualifiers
source                  1..178
                        mol_type = protein
                        organism = Escherichia coli
SEQUENCE: 414
MRRAFITGVF FLSEVEFSHE YWMRHALTLA KRAWDEREVP VGAVLVHNNR VIGEGWNRPI  60
GRHDPTAHAE IMALRQGGLV MQNYRLIDAT LYVTLEPCVM CAGAMIHSRI GRVVFGARDA  120
KTGAAGSLMD VLHHPGMNHR VEITEGILAD ECAALLSDFF RMRRQEIKAQ KKAQSSTD    178

SEQ ID NO: 415          moltype = AA  length = 160
FEATURE                 Location/Qualifiers
```

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```
source                  1..160
                        mol_type = protein
                        organism = Staphylococcus aureus
SEQUENCE: 415
MGSHMTNDIY FMTLAIEEAK KAAQLGEVPI GAIITKDDEV IARAHNLRET LQQPTAHAEH  60
IAIERAAKVL GSWRLEGCTL YVTLEPCVMC AGTIVMSRIP RVVYGADDPK GGCSGSLMNL  120
LQQSNFNHRA IVDKGVLKEA CSTLLTTFFK NLRANKKSTN                        160

SEQ ID NO: 416          moltype = AA  length = 161
FEATURE                 Location/Qualifiers
source                  1..161
                        mol_type = protein
                        organism = Bacillus subtilis
SEQUENCE: 416
MTQDELYMKE AIKEAKKAEE KGEVPIGAVL VINGEIIARA HNLRETEQRS IAHAEMLVID  60
EACKALGTWR LEGATLYVTL EPCPMCAGAV VLSRVEKVVF GAFDPKGGCS GTLMNLLQEE  120
RFNHQAEVVS GVLEEECGGM LSAFFRELRK KKKAARKNLS E                      161

SEQ ID NO: 417          moltype = AA  length = 183
FEATURE                 Location/Qualifiers
source                  1..183
                        mol_type = protein
                        note = Salmonella typhimurium
                        organism = unidentified
SEQUENCE: 417
MPPAFITGVT SLSDVELDHE YWMRHALTLA KRAWDEREVP VGAVLVHNHR VIGEGWNRPI  60
GRHDPTAHAE IMALRQGGLV LQNYRLLDTT LYVTLEPCVM CAGAMVHSRI GRVVFGARDA  120
KTGAAGSLID VLHHPGMNHR VEIIEGVLRD ECATLLSDFF RMRRQEIKAL KKADRAEGAG  180
PAV                                                                183

SEQ ID NO: 418          moltype = AA  length = 164
FEATURE                 Location/Qualifiers
source                  1..164
                        mol_type = protein
                        organism = Shewanella putrefaciens
SEQUENCE: 418
MDEYWMQVAM QMAEKAEAAG EVPVGAVLVK DGQQIATGYN LSISQHDPTA HAEILCLRSA  60
GKKLENYRLL DATLYITLEP CAMCAGAMVH SRIARVVYGA RDEKTGAAGT VVNLLQHPAF  120
NHQVEVTSGV LAEACSAQLS RFFKRRRDEK KALKLAQRAQ QGIE                   164

SEQ ID NO: 419          moltype = AA  length = 173
FEATURE                 Location/Qualifiers
source                  1..173
                        mol_type = protein
                        organism = Haemophilus influenzae
SEQUENCE: 419
MDAAKVRSEF DEKMMRYALE LADKAEALGE IPVGAVLVDD ARNIIGEGWN LSIVQSDPTA  60
HAEIIALRNG AKNIQNYRLL NSTLYVTLEP CTMCAGAILH SRIKRLVFGA SDYKTGAIGS  120
RFHFFDDYKM NHTLEITSGV LAEECSQKLS TFFQKRREEK KIEKALLKSL SDK         173

SEQ ID NO: 420          moltype = AA  length = 161
FEATURE                 Location/Qualifiers
source                  1..161
                        mol_type = protein
                        note = Caulobacter crescentus
                        organism = unidentified
SEQUENCE: 420
MRTDESEDQD HRMMRLALDA ARAAAEAGET PVGAVILDPS TGEVIATAGN GPIAAHDPTA  60
HAEIAAMRAA AAKLGNYRLT DLTLVVTLEP CAMCAGAISH ARIGRVVFGA DDPKGGAVVH  120
GPKFFAQPTC HWRPEVTGGV LADESADLLR GFFRARRKAK I                      161

SEQ ID NO: 421          moltype = AA  length = 179
FEATURE                 Location/Qualifiers
source                  1..179
                        mol_type = protein
                        organism = Geobacter sulfurreducens
SEQUENCE: 421
MSSLKKTPIR DDAYWMGKAI REAAKAAARD EVPIGAVIVR DGAVIGRGHN LREGSNDPSA  60
HAEMIAIRQA ARRSANWRLT GATLYVTLEP CLMCMGAIIL ARLERVVFGC YDPKGGAAGS  120
LYDLSADPRL NHQVRLSPGV CQEECGTMLS DFFRDLRRRK KAKATPALFI DERKVPPEP   179

SEQ ID NO: 422          moltype = AA  length = 167
FEATURE                 Location/Qualifiers
REGION                  1..167
                        note = Synthetic
source                  1..167
```

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```
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 422
MSEVEFSHEY WMRHALTLAK RAWDEREVPV GAVLVHNNRV IGEGWNRPIG RHDPTAHAAI  60
MALRQGGLVM QNYRLIDATL YVTLEPCVMC AGAMIHSRIG RVVFGARDAK TGAAGSLMDV  120
LHHPGMNHRV EITEGILADE CAALLSDFFR MRRQEIKAQK KAQSSTD                167

SEQ ID NO: 423        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 423
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 424        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 424
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGWRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 425        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 425
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRWAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 426        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 426
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRQAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 427        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 427
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGFRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 428        moltype = AA  length = 167
FEATURE               Location/Qualifiers
REGION                1..167
                      note = Synthetic
source                1..167
                      mol_type = protein
                      organism = synthetic construct
SEQUENCE: 428
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
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MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGQRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 429         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 429
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNRAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGMRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 430         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 430
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNFAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 431         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 431
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNWAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 432         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 432
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNQAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 433         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 433
MSEVEFSHEY WMRHALTLAK RARDEREVPV GAVLVLNNRV IGEGWNMAIG LHDPTAHAEI  60
MALRQGGLVM QNYRLIDATL YVTFEPCVMC AGAMIHSRIG RVVFGVRNAK TGAAGSLMDV  120
LHYPGMNHRV EITEGILADE CAALLCYFFR MPRQVFNAQK KAQSSTD                167

SEQ ID NO: 434         moltype = AA  length = 167
FEATURE                Location/Qualifiers
REGION                 1..167
                       note = Synthetic
source                 1..167
                       mol_type = protein
                       organism = synthetic construct
SEQUENCE: 434
MSEVEFSHEY WMRHALTLAK RAWDEREVPV GAVLVHNNRV IGEGWNRPIG RHDPTAHAQI  60
MALRQGGLVM QNYRLIDATL YVTLEPCVMC AGAMIHSRIG RVVFGARDAK TGAAGSLMDV  120
LHHPGMNHRV EITEGILADE CAALLSDFFR MRRQEIKAQK KAQSSTD                167

SEQ ID NO: 435         moltype = DNA  length = 145
```

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```
FEATURE                 Location/Qualifiers
misc_feature            1..145
                        note = Synthetic
misc_difference         1..22
                        note = n is a, c, g, or t
source                  1..145
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 435
nnnnnnnnnn nnnnnnnnnn nngtcatagt tcccctgaga aatcagggtt actatgataa  60
gggctttctg cctaaggcag actgacccgc ggcgttgggg atcgcctgtc gcccgctttt  120
ggcgggcatt ccccatcctt ttttt                                        145

SEQ ID NO: 436          moltype = AA  length = 22
FEATURE                 Location/Qualifiers
REGION                  1..22
                        note = Synthetic
source                  1..22
                        mol_type = protein
                        organism = synthetic construct
SEQUENCE: 436
GGSSGSETPG TSESATPESG GS                                           22
```

1-137. (canceled)

138. A polynucleotide encoding a fusion protein comprising an amino acid sequence that is at least 85% identical to the amino acid sequence of any one of SEQ ID NOS: 80-81.

139. A vector comprising a polynucleotide of claim **138**.

140. (canceled)

141. A cell comprising the polynucleotide of claim **138**.

142. A cell comprising the vector of claim **139**.

143. (canceled)

144. A pharmaceutical composition comprising the polynucleotide of claim **138** and a pharmaceutically acceptable carrier.

145. A pharmaceutical composition comprising the vector of claim **139** and a pharmaceutically acceptable carrier.

146. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of any one of SEQ ID NOs: 80-81.

147. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of any one of SEQ ID NOS: 80-81.

148. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 96% identical to the amino acid sequence of any one of SEQ ID NOS: 80-81.

149. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 97% identical to the amino acid sequence of any one of SEQ ID NOs: 80-81.

150. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 98% identical to the amino acid sequence of any one of SEQ ID NOS: 80-81.

151. The polynucleotide of claim **138**, wherein the fusion protein comprises an amino acid sequence that is at least 99% identical to the amino acid sequence of any one of SEQ ID NOs: 80-81.

152. The polynucleotide of claim **138**, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 80.

153. The polynucleotide of claim **138**, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 81.

154. A method comprising expressing a fusion protein from the polynucleotide of claim **138**, wherein the fusion protein is bound to a guide RNA (gRNA) that targets the fusion protein to a nucleic acid molecule.

155. The method of claim **154**, wherein the nucleic acid molecule comprises a target sequence associated with a disease or disorder.

156. The method of claim **154**, wherein the nucleic acid molecule comprises a point mutation associated with a disease or disorder.

157. The method of claim **156**, wherein the activity of the fusion protein results in a correction of the point mutation.

158. The method of claim **154**, wherein the contacting is performed in vivo in a subject.

159. The method of claim **154**, wherein the contacting is performed in vitro.

* * * * *