

METHODS AND COMPOSITIONS FOR MODULATING A GENOME

RELATED APPLICATIONS

This application claims priority to U.S. Serial No.: 63/241,931 filed September 8, 2021, the
5 entire contents of each of which is incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted
electronically in XML format and is hereby incorporated by reference in its entirety. Said XML
10 copy, created on September 8, 2021, is named V2065-7020WO_SL.xml and is 11,288,576 bytes
in size.

BACKGROUND

Integration of a nucleic acid of interest into a genome occurs at low frequency and with
15 little site specificity, in the absence of a specialized protein to promote the insertion event. Some
existing approaches, like CRISPR/Cas9, are more suited for small edits that rely on host repair
pathways, and are less effective at integrating longer sequences. Other existing approaches, like
Cre/loxP, require a first step of inserting a loxP site into the genome and then a second step of
inserting a sequence of interest into the loxP site. There is a need in the art for improved
20 compositions (e.g., proteins and nucleic acids) and methods for inserting, altering, or deleting
sequences of interest in a genome.

SUMMARY OF THE INVENTION

This disclosure relates to novel compositions, systems and methods for altering a genome
25 at one or more locations in a host cell, tissue or subject, *in vivo* or *in vitro*. In particular, the
invention features compositions, systems and methods for inserting, altering, or deleting
sequences of interest in a host genome. For example, the disclosure provides systems that are
capable of modulating (e.g., inserting, altering, or deleting sequences of interest) gene activity
and methods of treating disease by administering one or more such systems to alter a genomic
30 sequence at a nucleotide to correct a pathogenic mutation causing the disease.

Features of the compositions or methods can include one or more of the following enumerated embodiments.

1. A gene modifying polypeptide comprising:
a DNA binding domain (DBD) that binds to a target nucleic acid sequence and
5 a polymerase (Pol) domain of Table 1 or 23, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto;
wherein the DBD is heterologous to the Pol domain; and
a linker disposed between the Pol domain and the DBD.
2. A gene modifying polypeptide comprising:
10 a Cas domain (e.g., a Cas nickase domain, e.g., a Cas9 nickase domain);
a polymerase (Pol) domain of Table 1 or 23, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto, wherein the Pol domain is C-terminal of the Cas domain; and
a linker disposed between the Pol domain and the Cas domain.
- 15 3. The gene modifying polypeptide of embodiment 1 or 2, wherein the linker has a sequence from Table 6, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.
- 20 4. The gene modifying polypeptide of preceding embodiments, wherein the Pol domain has a sequence with at least 90% identity to the Pol domain of Table 1 or 23.
5. The gene modifying polypeptide of any of the preceding embodiments, wherein the Pol domain has a sequence with at least 95% identity to the Pol domain of Table 1 or 23.
- 25 6. The gene modifying polypeptide of any of the preceding embodiments, wherein the Pol domain has a sequence with at least 98% identity to the Pol domain of Table 1 or 23.
7. The gene modifying polypeptide of any of the preceding embodiments, wherein the Pol
30 domain has a sequence with at least 99% identity to the Pol domain of Table 1 or 23.

8. The gene modifying polypeptide of any of the preceding embodiments, wherein the Pol domain has a sequence with 100% identity to the Pol domain of Table 1 or 23.
- 5 9. The gene modifying polypeptide of any of the preceding embodiments, wherein the linker has a sequence with at least 90% identity to the linker sequence from Table 6.
10. The gene modifying polypeptide of any of the preceding embodiments, wherein the linker has a sequence with at least 95% identity to the linker sequence from Table 6.
- 10 11. The gene modifying polypeptide of any of the preceding embodiments, wherein the linker has a sequence with at least 97% identity to the linker sequence from Table 6.
12. The gene modifying polypeptide of any of the preceding embodiments, wherein the
15 linker has a sequence with 100% identity to the linker sequence from Table 6.
13. The gene modifying polypeptide of any of the preceding embodiments, wherein the Cas domain comprises a sequence of Table 4, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity thereto.
- 20 14. The gene modifying polypeptide of any of the preceding embodiments, wherein the Cas domain is a Cas nickase domain.
15. The gene modifying polypeptide of any of the preceding embodiments, wherein the Cas
25 domain is a Cas9 nickase domain.
16. The gene modifying polypeptide of any of the preceding embodiments, wherein the Cas domain comprises an N863A mutation.
- 30 17. The gene modifying polypeptide of any of the preceding embodiments, which comprises an NLS, e.g., wherein the gene modifying polypeptide comprises two NLSs.

18. The gene modifying polypeptide of any of the preceding embodiments, which comprises an NLS N-terminal of the Cas9 domain.
- 5 19. The gene modifying polypeptide of any of the preceding embodiments, which comprises an NLS C-terminal of the Pol domain.
20. The gene modifying polypeptide of any of the preceding embodiments, which comprises a first NLS which is N-terminal of the Cas9 domain and a second NLS which is C-terminal of
10 the Pol domain.
21. A nucleic acid (e.g., DNA or RNA, e.g., mRNA) encoding the gene modifying polypeptide of any of the preceding embodiments.
- 15 22. A cell comprising the gene modifying polypeptide of any of embodiments 1-20 or the nucleic acid of embodiment 21.
23. A system comprising:
i) the gene modifying polypeptide of any of embodiments 1-20, and
20 ii) a template nucleic acid (e.g., a template RNA) that comprises:
a) a gRNA spacer that is complementary to a portion a target nucleic acid sequence;
b) a gRNA scaffold that binds the Cas domain of the gene modifying polypeptide;
c) a heterologous object sequence; and
25 d) a primer binding site sequence (PBS sequence).
24. The system of embodiment 23, wherein the template nucleic acid comprises RNA.
25. The system of embodiment 23 or 24, wherein the template nucleic acid comprises DNA.
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26. The system of any of embodiments 23-25, wherein the gRNA spacer and the gRNA scaffold comprise RNA.
27. The system of any of embodiments 23-26, wherein the heterologous object sequence
5 comprises DNA and PBS sequence comprise RNA.
28. The system of any of embodiments 23-26, wherein the heterologous object sequence and PBS sequence comprise DNA.
- 10 29. A method for modifying a target nucleic acid in a cell (e.g., a human cell), the method comprising contacting the cell with the system of any of embodiments 23-28, or nucleic acid encoding the same, thereby modifying the target nucleic acid.
30. A method for treating a subject having a disease or condition associated with a genetic defect, the method comprising:
- 15 administering to the subject a system, polypeptide, template RNA or DNA encoding the same of any of the preceding embodiments, thereby treating the subject having a disease or condition associated with a genetic defect.
31. The method of embodiment 30 wherein the disease or condition associated with a genetic
20 defect is an indication listed in any of Tables 12-15 and/or wherein the genetic defect is a defect in a gene listed in any of Tables 12-15.
32. The method of embodiment 30 or 31, wherein the subject is a human patient.

In one aspect, the disclosure relates to a system for modifying a gene comprising (a) a nucleic acid encoding a gene modifying polypeptide capable of target primed reverse
25 transcription, the polypeptide comprising (i) a polymerase (Pol) domain and (ii) a Cas9 nickase that binds DNA and has endonuclease activity, and (b) a template RNA, DNA, or a hybrid having both ribonucleotide and deoxyribonucleotide residues in the same strand comprising (i) a gRNA spacer that is complementary to a first portion of the target gene, (ii) a gRNA scaffold that binds the polypeptide, (iii) a heterologous object sequence comprising a mutation region to

modify the gene, and (iv) a primer binding site (PBS) sequence comprising at least 3, 4, 5, 6, 7, or 8 bases of 100% homology to a target DNA strand at the 3' end of the template RNA.

The gRNA spacer may comprise at least 15 bases of 100% homology to the target DNA at the 5' end of the template RNA. The template RNA may further comprise a PBS sequence
5 comprising at least 5 bases of at least 80% homology to the target DNA strand. The template RNA may comprise one or more chemical modifications.

The domains of the gene modifying polypeptide may be joined by a peptide linker. The polypeptide may comprise one or more peptide linkers. The gene modifying polypeptide may further comprise a nuclear localization signal. The polypeptide may comprise more than one
10 nuclear localization signal, e.g., multiple adjacent nuclear localization signals or one or more nuclear localization signals in different regions of the polypeptide, e.g., one or more nuclear localization signals in the N-terminus of the polypeptide and one or more nuclear localization signals in the C-terminus of the polypeptide. The nucleic acid encoding the gene modifying polypeptide may encode one or more intein domains.

15 Introduction of the system into a target cell may result in insertion of at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 500, or 1000 base pairs of exogenous DNA. Introduction of the system into a target cell may result in deletion, wherein the deletion is less than 2, 3, 4, 5, 10, 50, or 100 base pairs of genomic DNA, upstream or downstream of the insertion. Introduction of the system into a target cell may result
20 in substitution, e.g., substitution of 1, 2, or 3 nucleotides, e.g., consecutive nucleotides.

The heterologous object sequence may be at least 5, 10, 25, 50, 100, 150, 200, 250, 300, 400, 500, 600, or 700 base pairs.

In one aspect, the disclosure relates to a pharmaceutical composition comprising the system described above and a pharmaceutically acceptable excipient or carrier, wherein the
25 pharmaceutically acceptable excipient or carrier is selected from the group consisting of a plasmid vector, a viral vector, a vesicle, and a lipid nanoparticle. In one aspect, the disclosure relates to a pharmaceutical composition comprising the system described above and multiple pharmaceutically acceptable excipients or carriers, wherein the pharmaceutically acceptable excipients or carriers are selected from the group consisting of a plasmid vector, a viral vector, a
30 vesicle, and a lipid nanoparticle, e.g., where the system described above is delivered by two

distinct excipients or carriers, e.g., two lipid nanoparticles, two viral vectors, or one lipid nanoparticle and one viral vector. The viral vector may be an adeno-associated virus (AAV).

In one aspect, the disclosure relates to a host cell (e.g., a mammalian cell, e.g., a human cell) comprising the system described above.

5 In one aspect, the disclosure relates to a method of correcting a mutation in the human gene in a cell, tissue or subject, the method comprising administering the system described above to the cell, tissue or subject. The system may be introduced *in vivo*, *in vitro*, *ex vivo*, or *in situ*. The nucleic acid of (a) may be integrated into the genome of the host cell. In some embodiments, the nucleic acid of (a) is not integrated into the genome of the host cell. In some
10 embodiments, the heterologous object sequence is inserted at only one target site in the host cell genome. The heterologous object sequence may be inserted at two or more target sites in the host cell genome, e.g., at the same corresponding site in two homologous chromosomes or at two different sites on the same or different chromosomes. The heterologous object sequence may encode a mammalian polypeptide, or a fragment or a variant thereof. The components of the
15 system may be delivered on 1, 2, 3, 4, or more distinct nucleic acid molecules. The system may be introduced into a host cell by electroporation or by using at least one vehicle selected from a plasmid vector, a viral vector, a vesicle, and a lipid nanoparticle.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a gene modifying system as described herein. The left hand diagram shows the
20 gene modifying polypeptide, which comprises a Cas nickase domain (e.g., spCas9 N863A) and a Pol domain which are linked by a linker. The right hand diagram shows the template nucleic acid which comprises, from 5' to 3', a gRNA spacer, a gRNA scaffold, a heterologous object sequence, and a primer binding site sequence (PBS sequence). The heterologous object sequence can comprise a mutation region that comprises one or more sequence differences
25 relative to the target site. The heterologous object sequence can also comprise a pre-edit homology region and a post-edit homology region, which flank the mutation region. Without wishing to be bound by theory, it is thought that the gRNA spacer of the template nucleic acid binds to the second strand of a target site in the genome, and the gRNA scaffold of the template nucleic acid binds to the gene modifying polypeptide, e.g., localizing the gene modifying
30 polypeptide to the target site in the genome. It is thought that the Cas domain of the gene modifying polypeptide nicks the target site (e.g., the first strand of the target site), e.g., allowing

the PBS sequence to bind to a sequence adjacent to the site to be altered on the first strand of the target site. It is thought that the Pol domain of the gene modifying polypeptide uses the first strand of the target site that is bound to the complementary sequence comprising the PBS sequence of the template nucleic acid as a primer and the heterologous object sequence of the template nucleic acid as a template to, e.g., polymerize a sequence complementary to the heterologous object sequence. Without wishing to be bound by theory, it is thought that DNA polymerization can then proceed through the pre-edit homology region, then through the mutation region, and then through the post-edit homology region, thereby producing a DNA strand comprising a mutation specified by the heterologous object sequence.

10 **FIG. 2** is a diagram showing exemplary truncations of a human DNA polymerase theta.

FIGS. 3A-3B are a series of graphs showing editing activity by Cas-Pol gene modifying polypeptides for the indicated template nucleic acid molecules in HEK293 cells (FIG. 3A) and U2OS cells (FIG. 3B).

15 DETAILED DESCRIPTION

Definitions

The term “expression cassette,” as used herein, refers to a nucleic acid construct comprising nucleic acid elements sufficient for the expression of the nucleic acid molecule of the instant invention.

20 A “gRNA spacer,” as used herein, refers to a portion of a nucleic acid that has complementarity to a target nucleic acid and can, together with a gRNA scaffold, target a Cas protein to the target nucleic acid.

A “gRNA scaffold,” as used herein, refers to a portion of a nucleic acid that can bind a Cas protein and can, together with a gRNA spacer, target the Cas protein to the target nucleic acid. In some embodiments, the gRNA scaffold comprises a crRNA sequence, tetraloop, and tracrRNA sequence.

25 A “gene modifying polypeptide,” as used herein, refers to a polypeptide comprising a polymerase or retroviral reverse transcriptase, or a polypeptide comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to a polymerase or retroviral reverse transcriptase, which is capable of

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integrating a nucleic acid sequence (e.g., a sequence provided on a template nucleic acid) into a target DNA molecule (e.g., in a mammalian host cell, such as a genomic DNA molecule in the host cell). In some embodiments, the gene modifying polypeptide is capable of integrating the sequence substantially without relying on host machinery. In some embodiments, the gene
5 modifying polypeptide integrates a sequence into a random position in a genome, and in some embodiments, the gene modifying polypeptide integrates a sequence into a specific target site. In some embodiments, a gene modifying polypeptide includes one or more domains that, collectively, facilitate 1) binding the template nucleic acid, 2) binding the target DNA molecule, and 3) facilitate integration of the at least a portion of the template nucleic acid into the target
10 DNA. Gene modifying polypeptides include both naturally occurring polypeptides as well as engineered variants of the foregoing, e.g., having one or more amino acid substitutions to the naturally occurring sequence. Gene modifying polypeptides also include heterologous constructs, e.g., where one or more of the domains recited above are heterologous to each other, whether through a heterologous fusion (or other conjugate) of otherwise wild-type domains, as
15 well as fusions of modified domains, e.g., by way of replacement or fusion of a heterologous sub-domain or other substituted domain. Exemplary gene modifying polypeptides, and systems comprising them and methods of using them, that can be used in the methods provided herein are described, e.g., in PCT/US2021/020948, which is incorporated herein by reference with respect to gene modifying polypeptides that comprise a retroviral reverse transcriptase domain. In some
20 embodiments, a gene modifying polypeptide integrates a sequence into a gene. In some embodiments, a gene modifying polypeptide integrates a sequence into a sequence outside of a gene. A “gene modifying system,” as used herein, refers to a system comprising a gene modifying polypeptide and a template nucleic acid.

The term “domain” as used herein refers to a structure of a biomolecule that contributes
25 to a specified function of the biomolecule. A domain may comprise a contiguous region (e.g., a contiguous sequence) or distinct, non-contiguous regions (e.g., non-contiguous sequences) of a biomolecule. Examples of protein domains include, but are not limited to, an endonuclease domain, a DNA binding domain, a polymerase (Pol) domain, a recruitment domain, a reverse transcription domain; an example of a domain of a nucleic acid is a regulatory domain, such as a
30 transcription factor binding domain. In some embodiments, a domain (e.g., a Cas domain) can

comprise two or more smaller domains (e.g., a DNA binding domain and an endonuclease domain).

As used herein, the term “exogenous,” when used with reference to a biomolecule (such as a nucleic acid sequence or polypeptide) means that the biomolecule was introduced into a host genome, cell or organism by the hand of man. For example, a nucleic acid that is added into an existing genome, cell, tissue or subject using recombinant DNA techniques or other methods is exogenous to the existing nucleic acid sequence, cell, tissue or subject.

As used herein, “first strand” and “second strand,” as used to describe the individual DNA strands of target DNA, distinguish the two DNA strands based upon which strand a Pol domain initiates polymerization, e.g., based upon where target primed synthesis initiates. The first strand refers to the strand of the target DNA upon which a Pol domain initiates polymerization, e.g., where target primed synthesis initiates. The second strand refers to the other strand of the target DNA. First and second strand designations do not describe the target site DNA strands in other respects; for example, in some embodiments the first and second strands are nicked by a polypeptide described herein, but the designations ‘first’ and ‘second’ strand have no bearing on the order in which such nicks occur.

A “genomic safe harbor site” (GSH site) is a site in a host genome that is able to accommodate the integration of new genetic material, e.g., such that the inserted genetic element does not cause significant alterations of the host genome posing a risk to the host cell or organism. A GSH site generally meets 1, 2, 3, 4, 5, 6, 7, 8 or 9 of the following criteria: (i) is located >300kb from a cancer-related gene; (ii) is >300kb from a miRNA/other functional small RNA; (iii) is >50kb from a 5' gene end; (iv) is >50kb from a replication origin; (v) is >50kb away from any ultraconserved element; (vi) has low transcriptional activity (i.e. no mRNA +/- 25 kb); (vii) is not in a copy number variable region; (viii) is in open chromatin; and/or (ix) is unique, with 1 copy in the human genome. Examples of GSH sites in the human genome that meet some or all of these criteria include (i) the adeno-associated virus site 1 (AAVS1), a naturally occurring site of integration of AAV virus on chromosome 19; (ii) the chemokine (C-C motif) receptor 5 (CCR5) gene, a chemokine receptor gene known as an HIV-1 coreceptor; (iii) the human ortholog of the mouse Rosa26 locus; (iv) the ribosomal DNA (“rDNA”) locus. Additional GSH sites are known and described, e.g., in Pellenz et al. epub August 20, 2018 (<https://doi.org/10.1101/396390>).

The term “heterologous,” as used herein to describe a first element in reference to a second element means that the first element and second element do not exist in nature disposed as described. For example, a heterologous polypeptide, nucleic acid molecule, construct or sequence refers to (a) a polypeptide, nucleic acid molecule or portion of a polypeptide or nucleic acid molecule sequence that is not native to a cell in which it is expressed, (b) a polypeptide or nucleic acid molecule or portion of a polypeptide or nucleic acid molecule that has been altered or mutated relative to its native state, or (c) a polypeptide or nucleic acid molecule with an altered expression as compared to the native expression levels under similar conditions. For example, a heterologous regulatory sequence (e.g., promoter, enhancer) may be used to regulate expression of a gene or a nucleic acid molecule in a way that is different than the gene or a nucleic acid molecule is normally expressed in nature. In another example, a heterologous domain of a polypeptide or nucleic acid sequence (e.g., a DNA binding domain of a polypeptide or nucleic acid encoding a DNA binding domain of a polypeptide) may be disposed relative to other domains or may be a different sequence or from a different source, relative to other domains or portions of a polypeptide or its encoding nucleic acid. In certain embodiments, a heterologous nucleic acid molecule may exist in a native host cell genome, but may have an altered expression level or have a different sequence or both. In other embodiments, heterologous nucleic acid molecules may not be endogenous to a host cell or host genome but instead may have been introduced into a host cell by transformation (e.g., transfection, electroporation), wherein the added molecule may integrate into the host genome or can exist as extra-chromosomal genetic material either transiently (e.g., mRNA) or semi-stably for more than one generation (e.g., episomal viral vector, plasmid or other self-replicating vector).

As used herein, “insertion” of a sequence into a target site refers to the net addition of DNA sequence at the target site, e.g., where there are new nucleotides in the heterologous object sequence with no cognate positions in the unedited target site. In some embodiments, a nucleotide alignment of the PBS sequence and heterologous object sequence to the target nucleic acid sequence would result in an alignment gap in the target nucleic acid sequence.

As used herein, a “deletion” generated by a heterologous object sequence in a target site refers to the net deletion of DNA sequence at the target site, e.g., where there are nucleotides in the unedited target site with no cognate positions in the heterologous object sequence. In some embodiments, a nucleotide alignment of the PBS sequence and heterologous object sequence to

the target nucleic acid sequence would result in an alignment gap in the molecule comprising the PBS sequence and heterologous object sequence.

The term “inverted terminal repeats” or “ITRs” as used herein refers to AAV viral cis-elements named so because of their symmetry. These elements promote efficient multiplication of an AAV genome. It is hypothesized that the minimal elements for ITR function are a Rep-binding site (RBS; 5′-GCGCGCTCGCTCGCTC-3′ for AAV2) and a terminal resolution site (TRS; 5′-AGTTGG-3′ for AAV2) plus a variable palindromic sequence allowing for hairpin formation. According to the present invention, an ITR comprises at least these three elements (RBS, TRS, and sequences allowing the formation of a hairpin). In addition, in the present invention, the term “ITR” refers to ITRs of known natural AAV serotypes (e.g. ITR of a serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 AAV), to chimeric ITRs formed by the fusion of ITR elements derived from different serotypes, and to functional variants thereof. “Functional variant” refers to a sequence presenting a sequence identity of at least 80%, 85%, 90%, preferably of at least 95% with a known ITR and allowing multiplication of the sequence that includes said ITR in the presence of Rep proteins.

The term “mutation region,” as used herein, refers to a region in a template nucleic acid having one or more sequence difference relative to the corresponding sequence in a target nucleic acid. The sequence difference may comprise, for example, a substitution, insertion, frameshift, or deletion.

The term “mutated” when applied to nucleic acid sequences means that nucleotides in a nucleic acid sequence are inserted, deleted, or changed compared to a reference (e.g., native) nucleic acid sequence. A single alteration may be made at a locus (a point mutation), or multiple nucleotides may be inserted, deleted, or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid sequence. A nucleic acid sequence may be mutated by any method known in the art.

“Nucleic acid molecule” refers to both RNA and DNA molecules including, without limitation, complementary DNA (“cDNA”), genomic DNA (“gDNA”), and messenger RNA (“mRNA”), and also includes synthetic nucleic acid molecules, such as those that are chemically synthesized or recombinantly produced, such as RNA templates, as described herein. The nucleic acid molecule can be double-stranded or single-stranded, circular, or linear. If single-stranded, the nucleic acid molecule can be the sense strand or the antisense strand. Unless

otherwise indicated, and as an example for all sequences described herein under the general format “SEQ ID NO:,” “nucleic acid comprising SEQ ID NO:1” refers to a nucleic acid, at least a portion which has either (i) the sequence of SEQ ID NO:1, or (ii) a sequence complimentary to SEQ ID NO:1. The choice between the two is dictated by the context in which SEQ ID NO:1 is used. For instance, if the nucleic acid is used as a probe, the choice between the two is dictated by the requirement that the probe be complementary to the desired target. Nucleic acid sequences of the present disclosure may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more naturally occurring nucleotides with an analog, inter-nucleotide modifications such as uncharged linkages (for example, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (for example, phosphorothioates, phosphorodithioates, etc.), pendant moieties, (for example, polypeptides), intercalators (for example, acridine, psoralen, etc.), chelators, alkylators, and modified linkages (for example, alpha anomeric nucleic acids, etc.).

Also included are chemically modified bases (see, for example, Table 9, *infra*), backbones (see, for example, Table 10, *infra*), and modified caps (see, for example, Table 11, *infra*). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of a molecule, e.g., peptide nucleic acids (PNAs). Other modifications can include, for example, analogs in which the ribose ring contains a bridging moiety or other structure such as modifications found in “locked” nucleic acids (LNAs). In various embodiments, the nucleic acids are in operative association with additional genetic elements, such as tissue-specific expression-control sequence(s) (e.g., tissue-specific promoters and tissue-specific microRNA recognition sequences), as well as additional elements, such as inverted repeats (e.g., inverted terminal repeats, such as elements from or derived from viruses, e.g., AAV ITRs) and tandem repeats, inverted repeats/direct repeats, homology regions (segments with various degrees of homology to a target DNA), untranslated regions (UTRs) (5′, 3′, or both 5′ and 3′ UTRs), and various combinations of the foregoing. The nucleic acid elements of the systems provided by the invention can be provided in a variety of topologies, including single-stranded, double-stranded, circular, linear, linear with open ends, linear with

closed ends, and particular versions of these, such as doggybone DNA (dbDNA), closed-ended DNA (ceDNA).

As used herein, a “gene expression unit” is a nucleic acid sequence comprising at least one regulatory nucleic acid sequence operably linked to at least one effector sequence. A first
5 nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if the promoter or enhancer affects the transcription or expression of the coding sequence. Operably
10 linked DNA sequences may be contiguous or non-contiguous. Where necessary to join two protein-coding regions, operably linked sequences may be in the same reading frame.

The terms “host genome” or “host cell,” as used herein, refer to a cell and/or its genome into which protein and/or genetic material has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell and/or genome, but to the progeny of such a cell and/or the genome of the progeny of such a cell. Because certain
15 modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein. A host genome or host cell may be an isolated cell or cell line grown in culture, or genomic material isolated from such a cell or cell line, or may be a host cell or host genome which composing living tissue or an organism. In
20 some instances, a host cell may be an animal cell or a plant cell, e.g., as described herein. In certain instances, a host cell may be a mammalian cell, a human cell, avian cell, reptilian cell, bovine cell, horse cell, pig cell, goat cell, sheep cell, chicken cell, or turkey cell. In certain instances, a host cell may be a corn cell, soy cell, wheat cell, or rice cell.

As used herein, “operative association” describes a functional relationship between two
25 nucleic acid sequences, such as a 1) promoter and 2) a heterologous object sequence, and means, in such example, the promoter and heterologous object sequence (e.g., a gene of interest) are oriented such that, under suitable conditions, the promoter drives expression of the heterologous object sequence. For instance, a template nucleic acid carrying a promoter and a heterologous object sequence may be single-stranded, e.g., either the (+) or (-) orientation. An “operative
30 association” between the promoter and the heterologous object sequence in this template means that, regardless of whether the template nucleic acid will be transcribed in a particular state,

when it is in the suitable state (e.g., is in the (+) orientation, in the presence of required catalytic factors, and NTPs, etc.), it is accurately transcribed. Operative association applies analogously to other pairs of nucleic acids, including other tissue-specific expression control sequences (such as enhancers, repressors and microRNA recognition sequences), IR/DR, ITRs, UTRs, or
5 homology regions and heterologous object sequences or sequences encoding a retroviral RT domain.

The term “primer binding site sequence” or “PBS sequence,” as used herein, refers to a portion of a template nucleic acid capable of binding to a region comprised in a target nucleic acid sequence. In some instances, a PBS sequence is a nucleic acid sequence comprising at least
10 3, 4, 5, 6, 7, or 8 bases with 100% identity to the region comprised in the target nucleic acid sequence. In some embodiments the primer region comprises at least 5, 6, 7, 8 bases with 100% identity to the region comprised in the target nucleic acid sequence. Without wishing to be bound by theory, in some embodiments when a template nucleic acid comprises a PBS sequence and a heterologous object sequence, the PBS sequence binds to a region comprised in a target
15 nucleic acid sequence, allowing a Pol domain to use that region as a primer for DNA polymerization, and to use the heterologous object sequence as a template.

As used herein, a “stem-loop sequence” refers to a nucleic acid sequence (e.g., RNA sequence) with sufficient self-complementarity to form a stem-loop, e.g., having a stem comprising at least two (e.g., 3, 4, 5, 6, 7, 8, 9, or 10) base pairs, and a loop with at least three
20 (e.g., four) base pairs. The stem may comprise mismatches or bulges.

As used herein, a “tissue-specific expression-control sequence” means nucleic acid elements that increase or decrease the level of a transcript comprising the heterologous object sequence in a target tissue in a tissue-specific manner, e.g., preferentially in on-target tissue(s), relative to off-target tissue(s). In some embodiments, a tissue-specific expression-control
25 sequence preferentially drives or represses transcription, activity, or the half-life of a transcript comprising the heterologous object sequence in the target tissue in a tissue-specific manner, e.g., preferentially in an on-target tissue(s), relative to an off-target tissue(s). Exemplary tissue-specific expression-control sequences include tissue-specific promoters, repressors, enhancers, or combinations thereof, as well as tissue-specific microRNA recognition sequences. Tissue
30 specificity refers to on-target (tissue(s) where expression or activity of the template nucleic acid is desired or tolerable) and off-target (tissue(s) where expression or activity of the template

nucleic acid is not desired or is not tolerable). For example, a tissue-specific promoter drives expression preferentially in on-target tissues, relative to off-target tissues. In contrast, a microRNA that binds the tissue-specific microRNA recognition sequences is preferentially expressed in off-target tissues, relative to on-target tissues, thereby reducing expression of a template nucleic acid in off-target tissues. Accordingly, a promoter and a microRNA recognition sequence that are specific for the same tissue, such as the target tissue, have contrasting functions (promote and repress, respectively, with concordant expression levels, i.e., high levels of the microRNA in off-target tissues and low levels in on-target tissues, while promoters drive high expression in on-target tissues and low expression in off-target tissues) with regard to the transcription, activity, or half-life of an associated sequence in that tissue.

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	a) Polypeptide components of gene modifying systems
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	ii) Endonuclease domains and DNA binding domains
	(1) Gene modifying polypeptides comprising Cas domains
	(2) TAL Effectors and Zinc Finger Nucleases
	iii) Linkers
20	iv) Localization sequences for gene modifying systems
	v) Evolved Variants of Gene modifying Polypeptides and Systems
	vi) Inteins
	vii) Additional domains
	b) Template nucleic acids
25	i) gRNA spacer and gRNA scaffold
	ii) Heterologous object sequence
	iii) PBS sequence
	iv) Exemplary Template Sequences
	c) gRNAs with inducible activity
30	d) Circular RNAs and Ribozymes in Gene modifying Systems
	e) Target Nucleic Acid Site

- f) Second strand nicking
- 3) Production of Compositions and Systems
- 4) Applications
 - a) Therapeutic Applications
 - 5 b) Application to Plants
 - 5) Administration and Delivery
 - a) Tissue Specific Activity/Administration
 - i) Promoters
 - ii) microRNAs
 - 10 b) Viral vectors and components thereof
 - c) AAV Administration
 - d) Lipid Nanoparticles
 - 6) Kits, Articles of Manufacture, and Pharmaceutical Compositions
 - 7) Chemistry, Manufacturing, and Controls (CMC)

15 **Introduction**

This disclosure relates to compositions, systems, and methods for targeting, editing, modifying, or manipulating a DNA sequence (e.g., inserting a heterologous object sequence into a target site of a mammalian genome) at one or more locations in a DNA sequence in a cell, tissue or subject, e.g., *in vivo*, or *in vitro*. The heterologous object DNA sequence may include, e.g., a substitution, a deletion, an insertion, e.g., a coding sequence, a regulatory sequence, or a gene expression unit.

More specifically, the disclosure provides DNA polymerase (Pol) based systems for altering a genomic DNA sequence of interest, e.g., by inserting, deleting, or substituting one or more nucleotides into/from the sequence of interest.

Fusions of a Cas9-related functionality to a polymerase functionality may be used to drive modifications to genomic DNA. The polymerase functionality may be, e.g., a DNA polymerase that synthesizes DNA from a nucleic acid template. The nucleic acid template may be, for example, DNA or RNA. In the case of a DNA polymerase that can use an RNA template, e.g., an RNA-dependent DNA polymerase, e.g., a reverse transcriptase, the gene modifying polypeptide component may be provided with a template RNA such as described above. One such example is DNA polymerase θ (encoded by POLQ, the polypeptide product of which may

be referred to herein as “POLQ” or “Polθ”), a eukaryotic DNA polymerase that has been shown to use either DNA or RNA as a template. Chandramouly et al. 2021 (DOI: 10.1126/sciadv.abf1771). A Cas9 functionality fused (optionally through a linker) to POLQ (or a component of POLQ) can therefore be used as a driver for genome modification when
5 administered to an organism or to cells with a template RNA that targets the genomic site desired for modification (via the gRNA spacer), recruits the Cas9 functionality (via the gRNA scaffold), and primes and templates DNA synthesis (via the template RNA).

The disclosure provides, in part, gene modifying systems comprising a gene modifying polypeptide component and a template nucleic acid (e.g., template RNA) component. In some
10 embodiments, a gene modifying system can be used to introduce an alteration into a target site in a genome. In some embodiments, the gene modifying polypeptide component comprises a writing domain (e.g., a reverse transcriptase domain), a DNA-binding domain, and an endonuclease domain (e.g., nickase domain). In some embodiments, the template nucleic acid (e.g., template RNA) comprises a sequence (e.g., a gRNA spacer) that binds a target site in the
15 genome (e.g., that binds to a second strand of the target site), a sequence (e.g., a gRNA scaffold) that binds the gene modifying polypeptide component, a heterologous object sequence, and a PBS sequence. Without wishing to be bound by theory, it is thought that the template nucleic acid (e.g., template RNA) binds to the second strand of a target site in the genome, and binds to the gene modifying polypeptide component (e.g., localizing the polypeptide component to the
20 target site in the genome). It is thought that the endonuclease (e.g., nickase) of the gene modifying polypeptide component cuts the target site (e.g., the first strand of the target site), e.g., allowing the PBS sequence to bind to a sequence adjacent to the site to be altered on the first strand of the target site. It is thought that the writing domain (e.g., reverse transcriptase domain) of the polypeptide component uses the first strand of the target site that is bound to the
25 complementary sequence comprising the PBS sequence of the template nucleic acid as a primer and the heterologous object sequence of the template nucleic acid as a template to, e.g., polymerize a sequence complementary to the heterologous object sequence. Without wishing to be bound by theory, it is thought that selection of an appropriate heterologous object sequence can result in substitution, deletion, and/or insertion of one or more nucleotides at the target site.

Gene modifying systems

In some embodiments, a gene modifying system described herein comprises: (A) a gene modifying polypeptide or a nucleic acid encoding the gene modifying polypeptide, wherein the gene modifying polypeptide comprises (i) a reverse transcriptase domain, and either (x) an
5 endonuclease domain that contains DNA binding functionality or (y) an endonuclease domain and separate DNA binding domain; and (B) a template RNA. A gene modifying polypeptide, in some embodiments, acts as a substantially autonomous protein machine capable of integrating a template nucleic acid sequence into a target DNA molecule (e.g., in a mammalian host cell, such as a genomic DNA molecule in the host cell), substantially without relying on host machinery.
10 For example, the gene modifying protein may comprise a DNA-binding domain, a reverse transcriptase domain, and an endonuclease domain. In some embodiments, the DNA-binding function may involve an RNA component that directs the protein to a DNA sequence, e.g., a gRNA spacer. In other embodiments, the gene modifying polypeptide may comprise a reverse transcriptase domain and an endonuclease domain. The RNA template element of a gene
15 modifying system is typically heterologous to the gene modifying polypeptide element and provides an object sequence to be inserted (reverse transcribed) into the host genome. In some embodiments, the gene modifying polypeptide is capable of target primed reverse transcription. In some embodiments, the gene modifying polypeptide is capable of second-strand synthesis.

In some embodiments the gene modifying system is combined with a second polypeptide.
20 In some embodiments, the second polypeptide may comprise an endonuclease domain. In some embodiments, the second polypeptide may comprise a polymerase domain, e.g., a reverse transcriptase domain. In some embodiments, the second polypeptide may comprise a DNA-dependent DNA polymerase domain. In some embodiments, the second polypeptide aids in completion of the genome edit, e.g., by contributing to second-strand synthesis or DNA repair
25 resolution.

A functional gene modifying polypeptide can be made up of unrelated DNA binding, reverse transcription, and endonuclease domains. This modular structure allows combining of functional domains, e.g., dCas9 (DNA binding), MMLV reverse transcriptase (reverse transcription), FokI (endonuclease). In some embodiments, multiple functional domains may
30 arise from a single protein, e.g., Cas9 or Cas9 nickase (DNA binding, endonuclease).

In some embodiments, a gene modifying polypeptide includes one or more domains that, collectively, facilitate 1) binding the template nucleic acid, 2) binding the target DNA molecule, and 3) facilitate integration of the at least a portion of the template nucleic acid into the target DNA. In some embodiments, the gene modifying polypeptide is an engineered polypeptide that
 5 comprises one or more amino acid substitutions to a corresponding naturally occurring sequence. In some embodiments, the gene modifying polypeptide comprises two or more domains that are heterologous relative to each other, e.g., through a heterologous fusion (or other conjugate) of otherwise wild-type domains, or well as fusions of modified domains, e.g., by way of replacement or fusion of a heterologous sub-domain or other substituted domain. For instance,
 10 in some embodiments, one or more of: the RT domain is heterologous to the DBD; the DBD is heterologous to the endonuclease domain; or the RT domain is heterologous to the endonuclease domain.

In some embodiments, a template RNA molecule for use in the system comprises, from 5' to 3' (1) a gRNA spacer; (2) a gRNA scaffold; (3) heterologous object sequence (4) a primer
 15 binding site (PBS) sequence. In some embodiments:

- (1) Is a gRNA spacer of ~18-22 nt, e.g., is 20 nt
- (2) Is a gRNA scaffold comprising one or more hairpin loops, e.g., 1, 2, of 3 loops for associating the template with a Cas domain, e.g., a nickase Cas9 domain. In some
 20 embodiments, the gRNA scaffold comprises the sequence, from 5' to 3',
 GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTT
 GAAAAAGTGGGACCGAGTCGGTCC (SEQ ID NO: 4008).
- (3) In some embodiments, the heterologous object sequence is, e.g., 7-74, e.g., 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, or 70-80 nt or, 80-90 nt in length. In some embodiments, the first (most 5') base of the sequence is not C.
- 25 (4) In some embodiments, the PBS sequence that binds the target priming sequence after nicking occurs is e.g., 3-20 nt, e.g., 7-15 nt, e.g., 12-14 nt. In some embodiments, the PBS sequence has 40-60% GC content.

In some embodiments, a second gRNA associated with the system may help drive complete integration. In some embodiments, the second gRNA may target a location that is 0-
 30 200 nt away from the first-strand nick, e.g., 0-50, 50-100, 100-200 nt away from the first-strand nick. In some embodiments, the second gRNA can only bind its target sequence after the edit is

made, e.g., the gRNA binds a sequence present in the heterologous object sequence, but not in the initial target sequence.

In some embodiments, a gene modifying system described herein is used to make an edit in HEK293, K562, U2OS, or HeLa cells. In some embodiment, a gene modifying system is used
5 to make an edit in primary cells, e.g., primary cortical neurons from E18.5 mice.

In some embodiments, a gene modifying polypeptide as described herein comprises a reverse transcriptase or RT domain (e.g., as described herein) that comprises a MoMLV RT sequence or variant thereof. In embodiments, the MoMLV RT sequence comprises one or more mutations selected from D200N, L603W, T330P, T306K, W313F, D524G, E562Q, D583N,
10 P51L, S67R, E67K, T197A, H204R, E302K, F309N, L435G, N454K, H594Q, D653N, R110S, and K103L. In embodiments, the MoMLV RT sequence comprises a combination of mutations, such as D200N, L603W, and T330P, optionally further including T306K and/or W313F.

In some embodiments, an endonuclease domain (e.g., as described herein) comprises nCAS9, e.g., comprising the H840A mutation.

15 In some embodiments, the heterologous object sequence (e.g., of a system as described herein) is about 1-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, 800-900, 900-1000, or more, nucleotides in length.

In some embodiments, the RT and endonuclease domains are joined by a flexible linker, e.g., comprising the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSS
20 (SEQ ID NO: 4006).

In some embodiments, the endonuclease domain is N-terminal relative to the RT domain. In some embodiments, the endonuclease domain is C-terminal relative to the RT domain.

In some embodiments, the system incorporates a heterologous object sequence into a target site by TPRT, e.g., as described herein.

25 In some embodiments, a gene modifying polypeptide comprises a DNA binding domain. In some embodiments, a gene modifying polypeptide comprises an RNA binding domain. In some embodiments, the RNA binding domain comprises an RNA binding domain of B-box protein, MS2 coat protein, dCas, or an element of a sequence of a table herein. In some embodiments, the RNA binding domain is capable of binding to a template RNA with greater
30 affinity than a reference RNA binding domain.

In some embodiments, a gene modifying system is capable of producing an insertion into the target site of at least 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides (and optionally no more than 500, 400, 300, 200, or 100 nucleotides). In some embodiments, a gene modifying system is capable of producing an insertion into the target site of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 nucleotides (and optionally no more than 500, 400, 300, 200, or 100 nucleotides). In some embodiments, a gene modifying system is capable of producing an insertion into the target site of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 kilobases (and optionally no more than 1, 5, 10, or 20 kilobases). In some embodiments, a gene modifying system is capable of producing a deletion of at least 81, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nucleotides (and optionally no more than 500, 400, 300, or 200 nucleotides). In some embodiments, a gene modifying system is capable of producing a deletion of at least 81, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nucleotides (and optionally no more than 500, 400, 300, or 200 nucleotides). In some embodiments, a gene modifying system is capable of producing a deletion of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 nucleotides (and optionally no more than 500, 400, 300, or 200 nucleotides). In some embodiments, a gene modifying system is capable of producing a deletion of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 kilobases (and optionally no more than 1, 5, 10, or 20 kilobases). In some embodiments, a gene modifying system is capable of producing a substitution into the target site of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, or 100 or more nucleotides. In some embodiments, a gene modifying system is capable of producing a substitution in the target site of 1-2, 2-3, 3-4, 4-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 nucleotides.

In some embodiments, the substitution is a transition mutation. In some embodiments, the substitution is a transversion mutation. In some embodiments, the substitution converts an adenine to a thymine, an adenine to a guanine, an adenine to a cytosine, a guanine to a thymine, a guanine to a cytosine, a guanine to an adenine, a thymine to a cytosine, a thymine to an adenine, a thymine to a guanine, a cytosine to an adenine, a cytosine to a guanine, or a cytosine to a thymine.

In some embodiments, an insertion, deletion, substitution, or combination thereof, increases or decreases expression (e.g. transcription or translation) of a gene. In some embodiments, an insertion, deletion, substitution, or combination thereof, increases or decreases expression (e.g. transcription or translation) of a gene by altering, adding, or deleting sequences in a promoter or enhancer, e.g. sequences that bind transcription factors. In some embodiments, an insertion, deletion, substitution, or combination thereof alters translation of a gene (e.g. alters an amino acid sequence), inserts or deletes a start or stop codon, alters or fixes the translation frame of a gene. In some embodiments, an insertion, deletion, substitution, or combination thereof alters splicing of a gene, e.g. by inserting, deleting, or altering a splice acceptor or donor site. In some embodiments, an insertion, deletion, substitution, or combination thereof alters transcript or protein half-life. In some embodiments, an insertion, deletion, substitution, or combination thereof alters protein localization in the cell (e.g. from the cytoplasm to a mitochondria, from the cytoplasm into the extracellular space (e.g. adds a secretion tag)). In some embodiments, an insertion, deletion, substitution, or combination thereof alters (e.g. improves) protein folding (e.g. to prevent accumulation of misfolded proteins). In some embodiments, an insertion, deletion, substitution, or combination thereof, alters, increases, decreases the activity of a gene, e.g. a protein encoded by the gene.

Exemplary gene modifying polypeptides, and systems comprising them and methods of using them are described, e.g., in PCT/US2021/020948, which is incorporated herein by reference with respect to retroviral RT domains, including the amino acid and nucleic acid sequences therein.

Exemplary gene modifying polypeptides and retroviral RT domain sequences are also described, e.g., in International Application No. PCT/US21/20948 filed March 4, 2021, e.g., at Table 30, Table 31, and Table 44 therein; the entire application is incorporated by reference herein with respect to retroviral RTs, e.g., in said sequences and tables. Accordingly, a gene modifying polypeptide described herein may comprise an amino acid sequence according to any of the Tables mentioned in this paragraph, or a domain thereof (e.g., a retroviral RT domain), or a functional fragment or variant of any of the foregoing, or an amino acid sequence having at least 70%, 80%, 85%, 90%, 95%, or 99% identity thereto.

In some embodiments, a polypeptide for use in any of the systems described herein can be a molecular reconstruction or ancestral reconstruction based upon the aligned polypeptide sequence of multiple homologous proteins. In some embodiments, a reverse transcriptase domain for use in any of the systems described herein can be a molecular reconstruction or an ancestral reconstruction, or can be modified at particular residues, based upon alignments of reverse transcriptase domains from the same or different sources. A skilled artisan can, based on the Accession numbers provided herein, align polypeptides or nucleic acid sequences, e.g., by using routine sequence analysis tools as Basic Local Alignment Search Tool (BLAST) or CD-Search for conserved domain analysis. Molecular reconstructions can be created based upon sequence consensus, e.g. using approaches described in Ivics et al., Cell 1997, 501 – 510; Wagstaff et al., Molecular Biology and Evolution 2013, 88-99.

Polypeptide components of gene modifying systems

In some embodiments, the gene modifying polypeptide possesses the functions of DNA target site binding, template nucleic acid (e.g., RNA) binding, DNA target site cleavage, and template nucleic acid (e.g., RNA) writing, e.g., reverse transcription. In some embodiments, each function is contained within a distinct domain. In some embodiments, a function may be attributed to two or more domains (e.g., two or more domains, together, exhibit the functionality). In some embodiments, two or more domains may have the same or similar function (e.g., two or more domains each independently have DNA-binding functionality, e.g., for two different DNA sequences). In other embodiments, one or more domains may be capable of enabling one or more functions, e.g., a Cas9 domain enabling both DNA binding and target site cleavage. In some embodiments, the domains are all located within a single polypeptide. In some embodiments, a first domain is in one polypeptide and a second domain is in a second polypeptide. For example, in some embodiments, the sequences may be split between a first polypeptide and a second polypeptide, e.g., wherein the first polypeptide comprises a reverse transcriptase (RT) domain and wherein the second polypeptide comprises a DNA-binding domain and an endonuclease domain, e.g., a nickase domain. As a further example, in some embodiments, the first polypeptide and the second polypeptide each comprise a DNA binding domain (e.g., a first DNA binding domain and a second DNA binding domain). In some embodiments, the first and second polypeptide may be brought together post-translationally via a split-intein to form a single gene modifying polypeptide.

In some aspects, a gene modifying polypeptide described herein comprises (e.g., a system described herein comprises a gene modifying polypeptide that comprises): 1) a Cas domain (e.g., a Cas nickase domain, e.g., a Cas9 nickase domain); 2) a reverse transcriptase (RT) domain of Table D, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto, wherein the RT domain is C-terminal of the Cas domain; and a linker disposed between the RT domain and the Cas domain, wherein the linker has a sequence from the same row of Table D as the RT domain, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.

In some embodiments, the RT domain has a sequence with 100% identity to the RT domain of Table D and the linker has a sequence with 100% identity to the linker sequence from the same row of Table D as the RT domain. In some embodiments, the Cas domain comprises a sequence of Table 4, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity thereto. In some embodiments, the gene modifying polypeptide comprises an amino acid sequence according to any of SEQ ID NOs: 1-3332 in the sequence listing, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.

In some embodiments, the gene modifying polypeptide comprises a GG amino acid sequence between the Cas domain and the linker, an AG amino acid sequence between the RT domain and the second NLS, and/or a GG amino acid sequence between the linker and the RT domain. In some embodiments, the gene modifying polypeptide comprises a sequence of SEQ ID NO: 4000 which comprises the first NLS and the Cas domain, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity thereto. In some embodiments, the gene modifying polypeptide comprises a sequence of SEQ ID NO: 4001 which comprises the second NLS, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity thereto.

Exemplary N-terminal NLS-Cas9 domain

MPAAKRVKLDGGDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF
 DSGETAETRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHP
 IFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYLAHMIKFRGHFLIEGDLNPDNSDV
 DKLFIQLVQTYNQLFEEINPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLIALS
 LGLTPNFKSNFDLAEDAKLQLSKDITYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVN
 TEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFY
 KFIKPILEKMDGTEELLVVLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPFLKDNR

EKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKN
 LPNEKVLPHKSLLEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLK
 EDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDR
 EMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNF
 5 MQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIAKKGILQTVKVVDELVKVMGRHKPE
 NIVIMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRD
 MYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKARGKSDNVPSEEVVKKMKNYWRQ
 LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKL
 IREVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGDY
 10 KQYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
 RDFATVRKVLSPQVNIKKTEVQTTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAY
 SVLVVAKVEKGSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLE
 LENGKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEQKQFLVEQHKHYLDEI
 IEQISEFSKRVLADANLDKVL SAYNKHDKPIREQAENI IHLFTLTNLGAPAAFKYFDTTIDR
 15 KRYTSTKEVLDTLIHQSIITGLYETRIDLSQLGGDGG (SEQ ID NO: 4000)

Exemplary C-terminal sequence

AGKRTADGSEFEKRTADGSEFESPKKKAKVE (SEQ ID NO: 4001)

20 *Writing domain*

In certain aspects of the present invention, the writing domain of the gene modifying
 system utilizes a polymerase functionality to drive modifications to genomic DNA. The
 polymerase functionality may be, e.g., a DNA polymerase that synthesizes DNA from a nucleic
 acid template. The nucleic acid template may be, for example, DNA or RNA. In the case of a
 25 DNA polymerase that can use an RNA template, e.g., an RNA-dependent DNA polymerase, e.g.,
 a reverse transcriptase, the gene modifying polypeptide component may be provided with a
 template RNA such as described above. One such example is DNA polymerase θ (encoded by
 POLQ, the polypeptide product of which may be referred to herein as “POLQ” or “Pol θ ”), a
 eukaryotic DNA polymerase that has been shown to use either DNA or RNA as a template.
 30 Chandramouly et al. 2021 (DOI: 10.1126/sciadv.abf1771). A Cas9 functionality fused
 (optionally through a linker) to POLQ (or a component of POLQ) can therefore be used as a
 driver for genome modification when administered to an organism or to cells with a template
 RNA that targets the genomic site desired for modification (via the gRNA spacer), recruits the
 Cas9 functionality (via the gRNA scaffold), and primes and templates DNA synthesis (via the
 35 template RNA).

A DNA polymerase that uses DNA as a template may also be incorporated in a fusion with a Cas9 functionality to effect genome modification. In this case, the template nucleic acid is a fusion of an sgRNA and a DNA template, joined end-to-end to one another by a covalent bond or by a linker. A poltheta (or component thereof) can also function in this way, as poltheta can synthesize DNA from a DNA template. It is understood that embodiments referring to template RNAs, as described herein, can include template nucleic acids comprising ribonucleotides, or template nucleic acid comprising ribonucleotides and deoxyribonucleotides (e.g., a template RNA comprising one or more RNA regions coupled to one or more DNA regions.) In some embodiments, a gene modifying polypeptide described herein comprises a polymerase domain having an amino acid sequence according to Table 1, or a sequence having at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto. In some embodiments, a nucleic acid described herein encodes a polymerase domain having an amino acid sequence according to Table 1, or a sequence having at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.

It is understood that embodiments referring to reverse transcriptases or reverse transcriptase domains, as described herein, can include a polymerase as listed in Table 1.

Table 1: Exemplary Polymerases for use in genome engineering polypeptides.

Row	Pol	Sequence	Length
1	Eta (η)	MATGQDRVALVDMDCFFVQVEQRQNPHLRNPICAVVQYKSWKGGGIIAVSYEARAFGVTRSMWADDAKKLCPDLLLAQVRESRGKANLTKYREASVEVMEIMSRFVIERASIDEAYVDLTSVAVQERLQKLGGQIPISADLLPSTYIEGLPQGPTTAEETVQKEGMRKQGLFQWLDSLQIDNLTSPLDLQTVGAVIVEEMRAAIERETGFQCSAGISHNKVLAKLACGLNKPNRQTLVSHGSPVQLFSQMPIRKIRSLGGKLGASVIEILGIEYMGEITQFTESQLQSHFGEKNGSWLYAMCRGIEHDPVKPRQLPKTIGCSKNFPGKTALATREQVQVWLLQLAQELEERLTKDRNDNRVATQLVVSIRVQGDKRLSSLRRCALTRYDAHKMSHDAFTVIKNCNTSGIQTEWSPPLTMLFLCATKFSASAPSSSTDITSFLSSDPSSLPKVPVTSSEAKTQSGSPAVTATKKATTSLESFFQKAAERQKVKEASLSSLTAPTQAPMSNPSKPSLPFQTSQSTGTEPFFKQKSLLLKQKQLNNSVSSPQNPWSNCKALPNSLPTEYPCGVPVCEGVSKLEEESKATPAEMDLAHNSQSMHASSASKSVLEVTQKATPNPSLLAAEDQVPCCKGSLVPVWDMPEHMDYHFALELQKSFQLPHSSNPQVVSASHQSKRNPKSPLACTNKRPRPEGMQTLESFFKPLTH	713
2	Iota (ι)	MEKLGVEPEEEGGDDDEDAEAWAMELADVGAAASSQGVHDQVLPNASSRVIVHVDLDCFYAQVEMISNPELKDPLGVQQKYLVWTCNYEARKLGVKKLMNVRDAKEKCPQLVLNGEDLTRYREMSYKVTELLEEFSPVVERLGFDEFNFDLTEMVEKRLQQLQSDLSAVTSGHVYNNQNSINLLDVLHIRLLVGSQIAAEMREAMYNQLGLTGCAVASNKLAKLVSGVFPNQQTVLLPESCQHLIHSLNHIKEIPGIGYKTAKCLEALGINSVRDLQTFSPKILEKELGISVAQRIQKLSFGEDNSPVILSGPPQSFSEEDSFKKCSSEVEAKNKIEELASLLNRVCQDGRKPHTVRLIIRYSSEKHYGRESRQCPIPSHVIQKLGTGNYDVMTPMV/DILMKLFRNMVN/KMPFHLTLLSVCFCLNLKALNTAKGLIDYILMPSLSTTSRSGKHSFKMKDTHMEDFPKDKETNRDFLPSGRIESTRTRESPLDTTNFSKEKDINEFPLCSLPEGVDQEVFKLPVDIQEILSGKSREKFQKGSVSCPLHASRGVLSFFSKQM/QDIPINPRDHLSSSKQVSSVSPCEPGTSGFNSSSSSYMSSQKDYSLDNRLKDERISQGPKEPQGFHTNSNPVSAFHSFPNLQSEQLFSRNHTTDSHKQTVATDSHEGLTENREPDVDEKITFPSDIDPQVFYELPEAVQKELLAEWKRAGSDFHIGHK	740

Row	Pol	Sequence	Length
3	Kappa (κ)	MDSTKEKCDYKDDLLRMGLNDNKAGMEGLDKEKINKIMEATKGSIFYGNEKKEKQVNRQIENMMQKACITSQQLRKAQLQV DRFAMELEQSRNLNTIVHIDMDAFYAAVEMRDNPDKPIAVGSMMLSTSNYHARRFGVRAAMPGFIAKRLCPQLIIVPPNFDK YRAVSKEVKEILADYDPNFMAMSLDEAYLNITKHEERQNWPEDEKRRYFIKMGSSVENDNPGKEVNKLSEHERSISPLLFEESPSDV QPPGDPFQVNFEEQNPNQILQNSVVFGTSAQEVVKEIRFRIEQKTTLTASAGIAPNTMLAKVCSKDNKPNQYQILPNRQAVMDFIK DLPIRKVSGIGKVKTEKMLKALGIICTELYQQRALLSLFSETSWHYFLHISLGLSTHLTRDGERKSMSEVERTFSEINKAEQYSLCQ ELCSELAQDLQKERLKGRTVTIKLKNVNFVKTRASTVSSVSTAEEIFAIAKELLKTEIDAFPHPLRLMLGVRISFPNEEDRKHKQ QRSIIGFLQAGNQALSATECTLEKTDKDKFVKPLEMSHKKSFFDKRSEKRWSHQDTFKCEAVNQSFQTSQPFQVLKKMMNENLE ISENSDDCQILTCPVCFRAQGCISLEALNKHVDECLDGPSISENFKMFSCSHVSATKVNKKENVPASSLCEKQDYEAHPKIKEISSVD CIALVDITNSSKAESIDALSNKHSKEECSSLPKSFNIEHCHQNSSTVLENEVDGVSFRQYRQPYLCEVKTQALVCPVCONVEQ KTSDLTLFNVHVDVCLNKSFIQELRKDKFNPVNPQKESRSTGSSSGVQKAVTRTKRPLMTKYSTSKKIPNNPKHTLDIFFK	870
4	REV1	MRRGGWRKRAENDGWETWGGYMAAKVQKLEEQFRSDAAMQKDGTSSTIFSGVAIYVNGYTDPSAEELRKLMMHGGQYHVYYS RSKTHIATNLPNAKIKELGKEKVRPEWIVESIKAGRLSYIPYQLYTKQSSVQKGLSFNPVCRPEDPLPGPSNIAKQLNNRVNHIVK KIETENEVKVNGMNSWNEEDENDFSVDLEQTSPPGRKQNGIPHRGSTAIFNGHTPSSNGALKTDCLVPMVNSVASRLSPAFS QEEDKAEKSSDTRDCTLQQLQSTRNTDALRNPHTNSFSLSPHSNTKINGAHHSTVQGPSSTKSTSSVTSFKAAPSVPSKPS DCNFISNFYSRHLHHISMWKCELTEFVNTLQRQSGIFGREKLKMKMTGRSALVTDGDMSVLNSPRHQSCIMHVDMDCCFFVS VGIRNRPDLKGPVAVTSNRGTGRAPLRPGANPQLEWQYQNKILKGKAADIPDSSLWENPDSAGANGIDSVLSRAEIASCSYEAR QLGKNGMFFGHAKQLCPNLQAVPYDFHAYKEVAQTLYETLASYTHNIEAVSCDEALVDITEILAEKLTPEFANAVRMEIKDQTKC AASVIGSINILLARMATRAKAPDGQYHLKPEEVDDFIRGQVLVNLPGVGHSMESKLASLGIKTCGDLQYMTMAKLQKEFGPKTGQM LYRFRGLDDRPVTEKERKSVSAEINYGIRFTQPKAEAFLLSSEIQRRLLEATGMKGKRLTKIMVRKPGAPVETAKFGGHGICD NIARTVTLQADTNAKIGKAMLMFMHTMKLNISDMRGVGIHVNLVPTNLNPSTCPSRPSVQSSHFSGSYSVRDVFQVQKAKKST EEEEKEVFRAADVLEISSASRTCTFLPPFAHLPTSPDTNKAESSGKWNLHTPVSVQSRNLNLSIEVSPSPQLDQSVLEALPPDLRE QVEQVCAVQQAESHGDKKKEPVNGCNTGILPQPVGTLLQIPEPQESNSDAGINLIALPAFSQVDPEVFAALPAELQRELKAAYDQR QRQGENSTHQSSASASVPKNPLHLKAHVKEKRNKKKTIGSPKRIQSPLNNKLLNSPAKTLPGACGSPQLIDGFLKHEGPPAEK PLEELASTSGVPLGSSLQSDPAGCVRPPAPNLGAVEFNDVKTLLREWITTISDPMEEIDILQVVKYCTDLIEEKDLEKLDLVIKYMKR LMQQSVSESVWNMAFDILDNVQVVLQQTYGSTLKV	1251
5	Beta (β)	MSKRKAPQETLNGGITDMLTELANFEKNVQAIHKYNAYRKAASVIAKYPHKIKSGAEAKKLPVGTGKIAEKIDEFLATGKLRKLEKIR QDDTSSSINFILTRVSGIGPSAARKFVDEGIKTLEDLRKNEDKLNHHQRIGLYKFGDFEKRIPREEMLMQMDIVLNEVKKVDSEYIATV CGSFRRAESSGDMDVLLTHPSFTSESTKQPKLLHQVVEQLQKVHFITDLSKGETKFMGVQCQLPSKNDEKEYPHRRDIRLIPKQDQ YYCGVLVYFTGSDIFNKNMRAHALEKGTINEYTRPLGVTGAGEPLPVDSEKIDIFYQWKYREPKDRSE	335
6	Lambda (λ)	MDPRGILKAFPRKQIHADASSKVLAKIPRREEGEEAEWLSLRAHVVRTGIGRARAELFEKQIVQHGGQLCPAQGGPVTHIVVDE GMDYERALLRLLPQLPPGAQLVKSAWLSLCLQERRLVDVAGFSIFIPSRYLDPHPQPSKAEQDASIPPGTHEALLQALSPPPPTTR PVSPQKAKEAPNTQAQPSISDEASDGEETQVSAADLEALISGHYPTSLGDCPEPSPAPAVLDKWVCAQPSQKATNHNHITKEL EVLAKAYSVQGDKWRLGYAKAINALKSFHKPVTSYQEAQCSIPGIGKMAEKIIEISGHLRKLHISESVPLVLELFSNIWGAGTKTA QMWWYQGFRLSDIRSQASLTQQAIGLKHYSDFLERMPREEATEIEQTVQKAAQAFNSGLLCVACGSYRKGKATCGDQVVDLITHP DGRSHRGIFSRLLDSLRLQEGFLTDDLVSQEENGQKQYLGVCRLPGPGRHRRLDIIVPYSEFACALLYFTGSAHFNRSMRALAK TKGMSLSEHALSTAVVRNTHGCKVGPGRVLPTEKDVFRLLGLPYREPAERDW	575
7	Mu (μ)	MLPKRRRARVGSPSGDAASSTPPSTRFPGVAIYVLEPRMGRSRRRAFLTGLARSKGFRVLDACSSEATHVMEETSAAEAVSWQER RMAAAPPGCTPPALLDISWLTESLGAGQPVPECRHRLEVAGPRKGPLSPAWMPAYACQRPTPLTHNTGLSEALEIAEAGFEG SEGRLLTFCRAASVLKALPSPVTTLSQLQLPHFGEHSSRVVQELLEHGVCSEEVERRRSERYQTMKLTQIFCVGVKTADRWYRE GLRTLDLREQPKLTQQQKAGLQHHQDLSTPVLRSVDALQVVEEAVGQALPGATVTLTGGFRRGKLGQHDVDFLITHPKEGQ EAGLLPRVMCRLDQGLILYHQHQHSCCESPTRLAQQSHMDAFERSFCIFRLPQPPGAAVGGSTRPCPSWKAVRVDLVAVPSVF PFALLGWTGSKLQRELRRFSRKEKGLWLNHGLFDPEQKTFQAAASEEDIFRHLGLELPPPEQRNA	494
8	TdT	MDPPRAHLSPPRKRPRQTGALMASSPDIFQDLVVFIEKKMGTTTTRAFRLMELARRKGFRVENELSDSVTHIVAENNSGSDVLE WLQAQKVQVSSQPELLDVSWLIECIRAGKPVEMTGKHLVWRDYSSTNPGPPKTPPIAVQKISQYACQRRRTLLNCCNQIFTDFA DILAENCEFRENEDESCVTFMRAASVLKSLPFTIISMKDTEGIPCLGSKVKGIIEEIEDGESSEVKAVLNDERYQSFKLFTSVFVGLKT	509

Row	Pol	Sequence	Length
		SEKWFRMGFRTLKSVRSDKSLKFTRMQKAGFLYYEDLVCVTRAEAEVSVLVKEAVWAFLPDAFVTMTGGFRRGKMGHVDVDF LITSPGSTEDDEQLLKVMNLWEKKGLLLYYDLVESTFEKRLPSRKVDALDHFQKCFILFKLPQRVDSQSSWQEGKTWKAIRVD LVLCYPYERRAFALLGWTGSRQFERDLRRYATHERKMILDNHALYDKTKRIFLKAEEEEIFAHLGLDYIEPWERN	
9	Theta (θ)	MNLLRRSGKRRRSESGSDSFGSGGSDSSASPQFLSGSVLSPPPGLGRCLKAAAAGECKPTVPDYERDKLLANWGLPKAVLEKYH SFGVKKMFEWQAECLLLQVLEGNLVYSAPTSAGKTLVAELLILKRVLEMRKKALFILPFVSAKEKKYQLQSLFQEVGKVDGYMG STSPSRHFSLDIAVCTIERANGLINRLIEENKMDLLGMVVDELHMLGDSHRGYLLELLTKICYITRKSASCQADLASSLNAVQIVG MSATLPNLELVASWLNALYHTDFRPVLLSVKVGNSIYSSMKLVREFEPMQVKCDEHVVSLCYETICDNHVSLLFCPSKKW CEKLADIAREFYNLHHQAEGLVKPSECPVILEQKELLEVMQDLRRLPSGLDSVLQKTVPWGAFHHAGLTFEERDIEGAFRQGLI RVLAATSLSSGVNLPARRVIPTIFGGRPLDILTYKQMVGRAGRKVDTVGESILICKNSEKSGIALQGSLLKPVRSCLQRREGEE VTGSMIRAILEIVGVASTSQDMHTYAACTFLAASMEGKQKQIRNQESVQLGAIEACVMWLLNEFIQISTEASDGTGKVVHPTH LGSATLSSSLSPADTLDFADLQRAMKGFVLENDLHILYLVTPMFEDWTIDWYRFFCLWEKLPTSMKRVAVELVGVEEGLARC/KVG KVVARTERQHRQMAIHKRFFTSVLDDLISEVPLREINQKYGCRNGQIQSLQSSAAVYAGMITVFSNRLGWHNMELLLSQFQKRLTF GIQRELCDLVRVSLNARARVLASGFHTVADLARANIVEVILKNAVFPKSARKAVDEEEAVEERRNMRTIWTGRKGLTERE AAALVIEEARMLQQLVEMGVQWNPCCALLHSSTCSLTHSESEVKEHTFISQTKSSYKLTSSKNKSNITFSDSYIKHSPNIVQDLNKS EHTSSFNCNFCNQNGEHQTCISFRARKRASLDINKEKPGASQNEGKTSDDKKVVQTFSSQTKKAPLNFSEKMSRFSRFSWKRKHL KRSRDSPLKDSGACRIHLQGGTSLNPSLCEPFTLDEKTEFRNSGPFKNVSLSGKEKDNKTSFPLQKQNCNSWNITLNDNFVE HIVTGSQSKNVTQCATSVVSEKGRGVAEAEKINEVLQNGSKNQNVYMKHHDHPINQYLKQSQHEQTSTITKQKNIERQMPCEAV SSYINRDSNVTINCEIRIKLNTENKPSHFQALGDDISRTVSEVLPVLSAGAFSKSEGQHENFLNISRLQEKGTGYTTNKTNNHVS LVLCDPDEFSFYLDTQSEKIQQMATENAKLGAKDTNLAAGIMQKSLVQQNSMNSFQKECHIPFAEQHPLGATKIDHDLKTVGT QSSDSHGVDILTPESPIFHSPILEENGFLKKNESVTDSQLNSFLQGYQTQETVKPVILLIPQKRTPTGVEGECPLVPETSLNMS LLFDSFSDDYLVKEQLPDMQMKLEPLSEVTSNHFSDSLCLQEDLIKSNVNEQDTHQQLTCSNDESIIFSEMDSVQMVEALDNVDI FPVQEKNHVTVSPRALELSDPVLDEHHQGDQDGGQDERAEKSKLTGTRQNHFSWGSAGFDLSPGLQRILDKVSSPLENEKLS MTINFSSLRNKNTELNEEQEVISNLETKVQGISFSSNNEVSKIEMLNANHDETSSLLPRKESNIVDDNGLIPTPTIPTSASKLTFP GILETPVNPWKTNVLPQGESYLFGPSDDIKNHDLSPGSRNGFKDNSPISDTSFSLQLSQDGLQLTPASSSSSESIIIDVASDQNL TFIKEWCRCKRFSISLACEKIRSLTSSKTATIGSRFKQASSPQEIPIRDDGFPKGCDDTLVGLAVCWGGRDAYYFSLQKEQKHSEIS ASLVPPSLDPSLTLDKRMWYLQSLRKESDKESVVIYDFIQSYKILLSCGISLEQSYEDPKVACWLLDPDSQEPTLHSIVSFLPHE LPLLEGMTSQGIQSLGLNAGSEHSGRYRASVESILIFNSMNQLNSLLQKENLDVFRKVEPMSQYCLALLELNGIGFSTAECESQK HIMQAKLDAIETQAYQLAGHSFSTSSDDIAEVLLELKLPPNREMKNQGSKTLGSTRGIDNGRKLRLGRQFSTSKDVLNKLKALH PLPGLILEWRRITNAITKVVPFLQREKCLNPFGLMERIYVVSQSHATGRITTEPNIQNVPRDFEIKMPTLVGESPPSQAVGKGLLPM GRGKYKKGFSVNPQCAQMEERAADRGMPFSISMRHAFVFPFGGSILAADYSQLELRILAHLSHRRLIQVLNTGADVFRSIAEW KMIEPESVGDDLQQAQKQICYGIYGMGAKSLGEQMGKENDAAACYDSFKSRYTGINGFMETVKNCKRDGFVQTLGRRRYLP DNNPYRKAHAERQAINIVQGSAAADIVKIATVNIQKQLETFHSTFKSHGHREGMLQSDQTLGSRKRLQGMFCPIRGFFILQLHDEL LYEVAEDVVQVQVKNEMESAVKLSVKLVKVKIGASWGELKDFDV	2590
10	Nu (ν)	MENYEALVGFDLCNTPLSSVAQKIMSAMHSGDLVDSTWKGSTETMEVINKSSVKYSVQLEDRTQSPEKDKLSLRSQTSRGS KLSPQSFSVRLTDQLSADQKQKSSSLTSSCLIPQYNQEAASVLQKGHKRKHFLMENINNENKGSINLKRKHITYNNLSEKTSKQMA LEEDTDAEGLYNSGNSGALKKHFCIDIRHLDWAKSqliemLKQAAALVITVMTDGTGLGADQTPVSSVGRVIVLVKRAEGGH GCPDAPACGPVLEGFVSDPCYIIEHSAIWDQEQAHHQFARNVLFQTMKCKCPVCFNAKDFVRIVLQFFGNDGSGWKHVADFIG LDPRIAAWLDPSDATPSFEDLVEKYCEKSITVKNVSTYGNSSRNIVNQNVRENKLTLYRLTMDLCSKLKDYGLWQLFRTLELPLIL AVMESHAIVQNKEMEKTSAALLGARLKELEQEAHFVAGERFLITSNNQLREILFGKLKHLHLSQRNSLPRTGLQKYPSTSEAVLNALR DLHPLPKIILEYRQVHKIKSTFVDGLLACMKKGSISSTWNQGTGTGRLSAKHPNIQGISKHPIQITTPKNFKGKEDKILTISPRAMFVS SKGHTFLAADFSQIELRILHLSGDPELLKLFQESERDDVFSTLTSQWKDVPVEQVTHADREQTKKVVYAVVYGAGKERLAACL IQEAAQFLESFLQYKIKDFARAAIAQCHQTCVVSIMGRRRPLPRIHAHDQQLRAQAERQAVNFVVQGSAAADLCKLAMIHVFTAV AASHTLTARLVAQHDELLFEVEDPQIPECAALVRRTMESLEQVQALELQLQVPLKVSLSAGRSWGHVLPLQEAWGPPPGPCTES PSNSLAAPGSPASTQPPPLHFSPSFCL	900

Table 1a: Properties of polymerases listed in Table 1

Row	Family	Pol	Host	Gene Symbol	UniProt Accession	UniProt Name	Catalytic Subunit Mass (kDa)	Additional Subunits	Biological Pathway(s)	Template Nucleic Acid
1	Y	Eta (η)	Homo sapiens	POLH	Q9Y253	POLH_HUMAN	78	Monomer	TLS	
2	Y	Iota (ι)	Homo sapiens	POLI	Q9UNA4	POLI_HUMAN	80	Monomer	TLS	
3	Y	Kappa (κ)	Homo sapiens	POLK	Q9UBT6	POLK_HUMAN	76	Monomer	TLS	
4	Y	REV1	Homo sapiens	REV1	Q9UBZ9	REV1_HUMAN	138	Monomer	TLS	
5	X	Beta (β)	Homo sapiens	POLB	P06746	DPOLB_HUMAN	39	Monomer	BER	
6	X	Lambda (λ)	Homo sapiens	POLL	Q9UGP5	DPOLL_HUMAN	66	Monomer	BER, NHEJ, TLS	
7	X	Mu (μ)	Homo sapiens	POLM	Q9NP87	DPOLM_HUMAN	55	Monomer	NHEJ, V(D)J	
8	X	TdT	Homo sapiens	DNTT	P04053	TDT_HUMAN	56	Monomer	V(D)J	Template-independent
9	A	Theta (θ)	Homo sapiens	POLQ	Q75417	DPOLQ_HUMAN	290	Monomer	TLS, MMEJ, RTDR	DNA and/or RNA
10	A	Nu (ν)	Homo sapiens	POLN	Q7Z5Q5	DPOLN_HUMAN	100	Monomer	ICL Repair	

For biological pathways, TLS = translesion synthesis; BER = base-excision repair; NHEJ = non-homologous end joining; V(D)J = V(D)J recombination process; MMEJ = microhomology-mediated end joining; RTDR = RNA-templated DNA repair.

5

In certain aspects of the present invention, the writing domain of the gene modifying system possesses reverse transcriptase activity and is also referred to as a reverse transcriptase domain (a RT domain). In some embodiments, the RT domain comprises an RT catalytic portion and RNA-binding region (e.g., a region that binds the template RNA).

10 In some embodiments, a nucleic acid encoding the reverse transcriptase is altered from its natural sequence to have altered codon usage, e.g. improved for human cells. In some embodiments the reverse transcriptase domain is a heterologous reverse transcriptase from a retrovirus. In some embodiments, the RT domain comprising a gene modifying polypeptide has been mutated from its original amino acid sequence, e.g., has at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 20, 30, 40, 50, 60, 70, 80, 90, or 100 substitutions. In some embodiments, the RT domain is derived from the RT of a retrovirus, e.g., HIV-1 RT, Moloney Murine Leukemia Virus (MMLV) RT, avian myeloblastosis virus (AMV) RT, or Rous Sarcoma Virus (RSV) RT.

In some embodiments, the retroviral reverse transcriptase (RT) domain exhibits enhanced stringency of target-primed reverse transcription (TPRT) initiation, e.g., relative to an endogenous RT domain. In some embodiments, the RT domain initiates TPRT when the 3 nt in the target site immediately upstream of the first strand nick, e.g., the genomic DNA priming the RNA template, have at least 66% or 100% complementarity to the 3 nt of homology in the RNA

20

template. In some embodiments, the RT domain initiates TPRT when there are less than 5 nt mismatched (e.g., less than 1, 2, 3, 4, or 5 nt mismatched) between the template RNA homology and the target DNA priming reverse transcription. In some embodiments, the RT domain is modified such that the stringency for mismatches in priming the TPRT reaction is increased, e.g.,

5 wherein the RT domain does not tolerate any mismatches or tolerates fewer mismatches in the priming region relative to a wild-type (e.g., unmodified) RT domain. In some embodiments, the RT domain comprises a HIV-1 RT domain. In embodiments, the HIV-1 RT domain initiates lower levels of synthesis even with three nucleotide mismatches relative to an alternative RT domain (e.g., as described by Jamburuthugoda and Eickbush *J Mol Biol* 407(5):661-672 (2011);

10 incorporated herein by reference in its entirety). In some embodiments, the RT domain forms a dimer (e.g., a heterodimer or homodimer). In some embodiments, the RT domain is monomeric. In some embodiments, an RT domain, naturally functions as a monomer or as a dimer (e.g., heterodimer or homodimer). In some embodiments, an RT domain naturally functions as a monomer, e.g., is derived from a virus wherein it functions as a monomer. In embodiments, the

15 RT domain is selected from an RT domain from murine leukemia virus (MLV; sometimes referred to as MoMLV) (e.g., P03355), porcine endogenous retrovirus (PERV) (e.g., UniProt Q4VFZ2), mouse mammary tumor virus (MMTV) (e.g., UniProt P03365), Mason-Pfizer monkey virus (MPMV) (e.g., UniProt P07572), bovine leukemia virus (BLV) (e.g., UniProt P03361), human T-cell leukemia virus-1 (HTLV-1) (e.g., UniProt P03362), human foamy virus

20 (HFV) (e.g., UniProt P14350), simian foamy virus (SFV) (e.g., UniProt P23074), or bovine foamy/syncytial virus (BFV/BSV) (e.g., UniProt O41894), or a functional fragment or variant thereof (e.g., an amino acid sequence having at least 70%, 80%, 90%, 95%, or 99% identity thereto). In some embodiments, an RT domain is dimeric in its natural functioning. In some

embodiments, the RT domain is derived from a virus wherein it functions as a dimer. In

25 embodiments, the RT domain is selected from an RT domain from avian sarcoma/leukemia virus (ASLV) (e.g., UniProt A0A142BKH1), Rous sarcoma virus (RSV) (e.g., UniProt P03354), avian myeloblastosis virus (AMV) (e.g., UniProt Q83133), human immunodeficiency virus type I (HIV-1) (e.g., UniProt P03369), human immunodeficiency virus type II (HIV-2) (e.g., UniProt P15833), simian immunodeficiency virus (SIV) (e.g., UniProt P05896), bovine

30 immunodeficiency virus (BIV) (e.g., UniProt P19560), equine infectious anemia virus (EIAV) (e.g., UniProt P03371), or feline immunodeficiency virus (FIV) (e.g., UniProt P16088)

(Herschhorn and Hizi *Cell Mol Life Sci* 67(16):2717-2747 (2010)), or a functional fragment or variant thereof (e.g., an amino acid sequence having at least 70%, 80%, 90%, 95%, or 99% identity thereto). Naturally heterodimeric RT domains may, in some embodiments, also be functional as homodimers. In some embodiments, dimeric RT domains are expressed as fusion proteins, e.g., as homodimeric fusion proteins or heterodimeric fusion proteins. In some
5 embodiments, the RT function of the system is fulfilled by multiple RT domains (e.g., as described herein). In further embodiments, the multiple RT domains are fused or separate, e.g., may be on the same polypeptide or on different polypeptides.

 In some embodiments, a gene modifying system described herein comprises an integrase
10 domain, e.g., wherein the integrase domain may be part of the RT domain. In some embodiments, an RT domain (e.g., as described herein) comprises an integrase domain. In some embodiments, an RT domain (e.g., as described herein) lacks an integrase domain, or comprises an integrase domain that has been inactivated by mutation or deleted. In some embodiment, a gene modifying system described herein comprises an RNase H domain, e.g., wherein the RNase
15 H domain may be part of the RT domain. In some embodiments, the RNase H domain is not part of the RT domain and is covalently linked via a flexible linker. In some embodiments, an RT domain (e.g., as described herein) comprises an RNase H domain, e.g., an endogenous RNase H domain or a heterologous RNase H domain. In some embodiments, an RT domain (e.g., as described herein) lacks an RNase H domain. In some embodiments, an RT domain (e.g., as
20 described herein) comprises an RNase H domain that has been added, deleted, mutated, or swapped for a heterologous RNase H domain. In some embodiments, the polypeptide comprises an inactivated endogenous RNase H domain. In some embodiments, an endogenous RNase H domain from one of the other domains of the polypeptide is genetically removed such that it is not included in the polypeptide, e.g., the endogenous RNase H domain is partially or completely
25 truncated from the comprising domain. In some embodiments, mutation of an RNase H domain yields a polypeptide exhibiting lower RNase activity, e.g., as determined by the methods described in Kotewicz et al. *Nucleic Acids Res* 16(1):265-277 (1988) (incorporated herein by reference in its entirety), e.g., lower by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to an otherwise similar domain without the mutation. In some embodiments,
30 RNase H activity is abolished.

In some embodiments, an RT domain is mutated to increase fidelity compared to an otherwise similar domain without the mutation. For instance, in some embodiments, a YADD or YMDD motif in an RT domain (e.g., in a reverse transcriptase) is replaced with YVDD. In embodiments, replacement of the YADD or YMDD or YVDD results in higher fidelity in retroviral reverse transcriptase activity (e.g., as described in Jamburuthugoda and Eickbush J Mol Biol 2011; incorporated herein by reference in its entirety).

In some embodiments, a gene modifying polypeptide described herein comprises an RT domain having an amino acid sequence according to Table 2, or a sequence having at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto. In some embodiments, a nucleic acid described herein encodes an RT domain having an amino acid sequence according to Table 2, or a sequence having at least 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.

Table 2: Exemplary reverse transcriptase domains from retroviruses

RT Name	RT amino acid sequence
AVIRE_P03360	TAPLEEEYRLFLEAPIQNVTLLEQWKREIPKVAEINPPGLASTQAPIHVQLLSTALPVRVRQYPITLEAKRSLRETIRKFRAAGILRPVHSPWNTPLLPVRKSGTSEYRMVQDLREVNRVETIHPTVPNPYTLTLLSLLPPDRIMYSVLDLKDFAFFCIPLAPESQLIFAFEWADAEEGESGQLTWTRLPQGFKNSPTLFDEALNRDLQGFRLDHPVSLLQYVDDLLIAADTQAACLSATRDLLMTLAEGLYRVSGKKAQLCQEEVYTLGFKIHKGSRSLNSRQTALQIPVPKTKRQVREFLGTIGYCRWLWIPGFAELAQLPYAATRGNDPLVWGEKEEEAFQSLKALTQPPALALPSLDKPFQLFVEETSGAAKGVLTQALGPWKRPVAYLSKRLLDPVAAGWPRCLRAIAAAALLTREASKLTFGQDIEITSSHNLESLLRSPDKWLTNARITQYQVLLDPPRVRFKQTAALNPATLLPETD DTLPIHHCLD TLDLSTSTRPDLTDQPLAQAEATLFTDGSSYIRDGKRYAGAAVVTLD S V I W A E P L P I G T S A Q K A E I A L T K A E W S K D K S V N I Y T D S R Y A F A T L H V H G M I Y R E R G L L T A G G K A I K N A P E I L A L L T A V W L P K R V A V M H C K G H Q K D D A P T S T G N R R A D E V A R E V A I R P L S T Q A T I S
AVIRE_P03360_3mut	TAPLEEEYRLFLEAPIQNVTLLEQWKREIPKVAEINPPGLASTQAPIHVQLLSTALPVRVRQYPITLEAKRSLRETIRKFRAAGILRPVHSPWNTPLLPVRKSGTSEYRMVQDLREVNRVETIHPTVPNPYTLTLLSLLPPDRIMYSVLDLKDFAFFCIPLAPESQLIFAFEWADAEEGESGQLTWTRLPQGFKNSPTLFNEALNRDLQGFRLDHPVSLLQYVDDLLIAADTQAACLSATRDLLMTLAEGLYRVSGKKAQLCQEEVYTLGFKIHKGSRSLNSRQTALQIPVPKTKRQVREFLGTIGYCRWLWIPGFAELAQLPYAATRGNDPLVWGEKEEEAFQSLKALTQPPALALPSLDKPFQLFVEETSGAAKGVLTQALGPWKRPVAYLSKRLLDPVAAGWPRCLRAIAAAALLTREASKLTFGQDIEITSSHNLESLLRSPDKWLTNARITQYQVLLDPPRVRFKQTAALNPATLLPETD DTLPIHHCLD TLDLSTSTRPDLTDQPLAQAEATLFTDGSSYIRDGKRYAGAAVVTLD S V I W A E P L P I G T S A Q K A E I A L T K A E W S K D K S V N I Y T D S R Y A F A T L H V H G M I Y R E R G L L T A G G K A I K N A P E I L A L L T A V W L P K R V A V M H C K G H Q K D D A P T S T G N R R A D E V A R E V A I R P L S T Q A T I S
AVIRE_P03360_3mutA	TAPLEEEYRLFLEAPIQNVTLLEQWKREIPKVAEINPPGLASTQAPIHVQLLSTALPVRVRQYPITLEAKRSLRETIRKFRAAGILRPVHSPWNTPLLPVRKSGTSEYRMVQDLREVNRVETIHPTVPNPYTLTLLSLLPPDRIMYSVLDLKDFAFFCIPLAPESQLIFAFEWADAEEGESGQLTWTRLPQGFKNSPTLFNEALNRDLQGFRLDHPVSLLQYVDDLLIAADTQAACLSATRDLLMTLAEGLYRVSGKKAQLCQEEVYTLGFKIHKGSRSLNSRQTALQIPVPKTKRQVREFLGTIGYCRWLWIPGFAELAQLPYAATRGNDPLVWGEKEEEAFQSLKALTQPPALALPSLDKPFQLFVEETSGAAKGVLTQALGPWKRPVAYLSKRLLDPVAAGWPRCLRAIAAAALLTREASKLTFGQDIEITSSHNLESLLRSPDKWLTNARITQYQVLLDPPRVRFKQTAALNPATLLPETD DTLPIHHCLD TLDLSTSTRPDLTDQPLAQAEATLFTDGSSYIRDGKRYAGAAVVTLD S V I W A E P L P I G T S A Q K A E I A L T K A E W S K D K S V N I Y T D S R Y A F A T L H V H G M I Y R E R G L L T A G G K A I K N A P E I L A L L T A V W L P K R V A V M H C K G H Q K D D A P T S T G N R R A D E V A R E V A I R P L S T Q A T I S
BAEVM_P1027 2	TVSLQDEHRLFDIPVTTSLPDVWLQDFQAWAETGGGLGRACKQAPIIIDLKPTAVPVSIKQYPMSEAHMGIRQHIKFLFELGVLPRCSPWNTPLLPVKKPGTQDYRPVQDLREINKRTVDIHPTVPNPYNLLSTLKPDSYWTYVLDLKDFAFFCLPLAPQSQELFAFEWKDPERGISGQLTWTRLPQGFKNSPTLFDEALNRDLTDFRTQHPEVTLTLLQYVDDLLLAAPTAKACTQGRHLLGELGEKGYRASAKKAQICQTKVYLYGILSEGKRWLTGPRIETVARIPPPRNPREVREFLGTAGFCRLWIPGFAELAAPLYALTKESTPFTWQTEHLQAFELKKALLSAPALGLPDTSKPFTFLDERQGIAGVLTQKLGPKWKRPVAYLSKKLDPVAAGWPPCLRIMAATAMLVKDSAKLTGQPLTVTPHTLEAIVRQPPDRWITNARLTHYQALLDTRVQFGPPVTLNPATLLPVENQPSPHDCRQVLAETHGTREDLKDQELPDADHTWYTDGSSYLDGSTRRAGA AVVDGHNTIWAQSLPPGTSAQKAEIALTKALEL SKGK KANIYTD SRYAFATAHTHGSYERRGLTSEGKEIKNAEIIALLKALFLPQEVAIHCPCGHQKQDQDPVAVGNRQADRVARQAAMAEVLTATEPDNTSHIT
BAEVM_P1027 2_3mut	TVSLQDEHRLFDIPVTTSLPDVWLQDFQAWAETGGGLGRACKQAPIIIDLKPTAVPVSIKQYPMSEAHMGIRQHIKFLFELGVLPRCSPWNTPLLPVKKPGTQDYRPVQDLREINKRTVDIHPTVPNPYNLLSTLKPDSYWTYVLDLKDFAFFCLPLAPQSQELFAFEWKDPERGISGQLTWTRLPQGFKNSPTLFNEALNRDLTDFRTQHPEVTLTLLQYVDDLLLAAPTAKACTQGRHLLGELGEKGYRASAKKAQICQTKVYLYGILSEGKRWLTGPRIETVARIPPPRNPREVREFLGTAGFCRLWIPGFAELAAPLYALTKESTPFTWQTEHLQAFELKKALLSAPALGLPDTSKPFTFLDERQGIAGVLTQKLGPKWKRPVAYLSKKLDPVAAGWPPCLRIMAATAMLVKDSAKLTGQPLTVTPHTLEAIVRQPPDRWITNARLTHYQALLDTRVQFGPPVTLNPATLLPVENQPSPHDCRQVLAETHGTREDLKDQELPDADHTWYTDGSSYLDGSTRRAGA AVVDGHNTIWAQSLPPGTSAQKAEIALTKALEL SKGK KANIYTD SRYAFATAHTHGSYERRGLTSEGKEIKNAEIIALLKALFLPQEVAIHCPCGHQKQDQDPVAVGNRQADRVARQAAMAEVLTATEPDNTSHIT
BAEVM_P1027 2_3mut A	TVSLQDEHRLFDIPVTTSLPDVWLQDFQAWAETGGGLGRACKQAPIIIDLKPTAVPVSIKQYPMSEAHMGIRQHIKFLFELGVLPRCSPWNTPLLPVKKPGTQDYRPVQDLREINKRTVDIHPTVPNPYNLLSTLKPDSYWTYVLDLKDFAFFCLPLAPQSQELFAFEWKDPERGISGQLTWTRLPQGFKNSPTLFNEALNRDLTDFRTQHPEVTLTLLQYVDDLLLAAPTAKACTQGRHLLGELGEKGYRASAKKAQICQTKVYLYGILSEGKRWLTGPRIETVARIPPPRNPREVREFLGTAGFCRLWIPGFAELAAPLYALTKESTPFTWQTEHLQAFELKKALLSAPALGLPDTSKPFTFLDERQGIAGVLTQKLGPKWKRP

RT Name	RT amino acid sequence
	VAYLSKKLDPAAGWPPCLRMAATAMLVKDSAKLTGQPLTVTPHTLEAIVRQPPDRWITNARLTHYQALLDTRVQFGPPVTLNPATLLPVPEN QPSPHDCRQVLAETHGTREDLKDQELPDADHTWYTDGSSYLDGSTRRAGAAGVVDGHNTIWAQSLPPGTSQAQKAEIALTKALEL SKGKKANIYTD SRYAFATAHTHGSYERRGWL TSEGKEIKNAEIALLKALFLPQEVAIHCPGHQKQDPVAVGNRQADRVARQAAMAEVL TLATEPDNTSHIT
BLVAU_P2505_9	GVLDAAPSHIGLEHLPPPEVPQFPLNLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNAGAWRFVHDLRVNLTAKIPALSPGPPDLTAI PTHLPPIICLDLKDFAFFQIPVEDRFRSYFAFTLPTPGGLQPHRRFAWRVLPQGFINSFALFERALQEPLRQVSAAFSGSLLVSYMDILYVSPTEEQ LQCYQTMAAHLRDLGFQVASEKTRQTPSPVPFLGQMVMHERMVTYQSLPTLQISSPISLHQLQTVLGDQLQWVSRGTPPTRRPLQLLYSSLKPIDDP AIHLSPEQQQGAIELRQALSHNARSRYNEQEPLLAYVHLTRAGSTLVLFQKGAQFPLAYFQTPLTNDQASPWGLLLLLGCQYLQAQALSSYAKTILK YYHNLPKTSLDNWQSSSEDPVQELLQLWPQISSQGIQPPGPWKLTITRAEVFLTPQFSPEPIPAALCLFSDGAARRGAYCLWKDHLDFQAVPAP ESAQKGELAGLGLAGLAAAPPEPLNIWVDSKYL YSLRLTLVLGAWLQPDVPVSYALLYKSLLRHPAIFVGHVRSHSSASHPIASLNNYVDQL
BLVAU_P2505_9_2mut	GVLDAAPSHIGLEHLPPPEVPQFPLNLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNAGAWRFVHDLRVNLTAKIPALSPGPPDLTAI PTHLPPIICLDLKDFAFFQIPVEDRFRSYFAFTLPTPGGLQPHRRFAWRVLPQGFINSFALFQALQEPLRQVSAAFSGSLLVSYMDILYVSPTEEQ LQCYQTMAAHLRDLGFQVASEKTRQTPSPVPFLGQMVMHERMVTYQSLPTLQISSPISLHQLQTVLGDQLQWVSRGTPPTRRPLQLLYSSLKPIDDP AIHLSPEQQQGAIELRQALSHNARSRYNEQEPLLAYVHLTRAGSTLVLFQKGAQFPLAYFQTPLTNDQASPWGLLLLLGCQYLQAQALSSYAKTILK YYHNLPKTSLDNWQSSSEDPVQELLQLWPQISSQGIQPPGPWKLTITRAEVFLTPQFSPEPIPAALCLFSDGAARRGAYCLWKDHLDFQAVPAP ESAQKGELAGLGLAGLAAAPPEPLNIWVDSKYL YSLRLTLVLGAWLQPDVPVSYALLYKSLLRHPAIFVGHVRSHSSASHPIASLNNYVDQL
BLVJ_P03361	GVLDTTPSHIGLEHLPPPEVPQFPLNLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNAGAWRFVHDLRATNLTAKIPALSPGPPDLTAI PTHPPIICLDLKDFAFFQIPVEDRFRSYFTLPSPGGLQPHRRFAWRVLPQGFINSFALFERALQEPLRQVSAAFSGSLLVSYMDILYASPTTEEQ SQCYQALAAHLRDLGFQVASEKTRQTPSPVPFLGQMVMHEQIVTYQSLPTLQISSPISLHQLQAVLGDQLQWVSRGTPPTRRPLQLLYSSLKRRHDP AIQLSPEQLQGIAELRQALSHNARSRYNEQEPLLAYVHLTRAGSTLVLFQKGAQFPLAYFQTPLTNDQASPWGLLLLLGCQYLQQAALSSYAKPILK YYHNLPKTSLDNWQSSSEDPVQELLQLWPQISSQGIQPPGPWKLTITRAEVFLTPQFSPEPIPAALCLFSDGATGRGAYCLWKDHLDFQAVPAP ESAQKGELAGLGLAGLAAAPPEPLNIWVDSKYL YSLRLTWL GAWLQPDVPVSYALLYKSLLRHPAIFVGHVRSHSSASHPIASLNNYVDQL
BLVJ_P03361_2mut	GVLDTTPSHIGLEHLPPPEVPQFPLNLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNAGAWRFVHDLRATNLTAKIPALSPGPPDLTAI PTHPPIICLDLKDFAFFQIPVEDRFRSYFTLPSPGGLQPHRRFAWRVLPQGFINSFALFNRALQEPLRQVSAAFSGSLLVSYMDILYASPTTEEQ SQCYQALAAHLRDLGFQVASEKTRQTPSPVPFLGQMVMHEQIVTYQSLPTLQISSPISLHQLQAVLGDQLQWVSRGTPPTRRPLQLLYSSLKRRHDP AIQLSPEQLQGIAELRQALSHNARSRYNEQEPLLAYVHLTRAGSTLVLFQKGAQFPLAYFQTPLTNDQASPWGLLLLLGCQYLQQAALSSYAKPILK YYHNLPKTSLDNWQSSSEDPVQELLQLWPQISSQGIQPPGPWKLTITRAEVFLTPQFSPEPIPAALCLFSDGATGRGAYCLWKDHLDFQAVPAP ESAQKGELAGLGLAGLAAAPPEPLNIWVDSKYL YSLRLTWL GAWLQPDVPVSYALLYKSLLRHPAIFVGHVRSHSSASHPIASLNNYVDQL
BLVJ_P03361_2mutB	GVLDTTPSHIGLEHLPPPEVPQFPLNLERLQALQDLVHRSLEAGYISPWDGPGNNPVFPVRKPNAGAWRFVHDLRATNLTAKIPALSPGPPDLTAI PTHPPIICLDLKDFAFFQIPVEDRFRSYFTLPSPGGLQPHRRFAWRVLPQGFINSFALFQALQEPLRQVSAAFSGSLLVSYMDILYASPTTEEQ RSQCYQALAAHLRDLGFQVASEKTRQTPSPVPFLGQMVMHEQIVTYQSLPTLQISSPISLHQLQAVLGDQLQWVSRGTPPTRRPLQLLYSSLKRRHDP RAIQLSPEQLQGIAELRQALSHNARSRYNEQEPLLAYVHLTRAGSTLVLFQKGAQFPLAYFQTPLTNDQASPWGLLLLLGCQYLQQAALSSYAKPIL KYHNLPKTSLDNWQSSSEDPVQELLQLWPQISSQGIQPPGPWKLTITRAEVFLTPQFSPEPIPAALCLFSDGATGRGAYCLWKDHLDFQAVPAP ESAQKGELAGLGLAGLAAAPPEPLNIWVDSKYL YSLRLTWL GAWLQPDVPVSYALLYKSLLRHPAIFVGHVRSHSSASHPIASLNNYVDQL
FFV_O93209	MDLLKPLTVERKGVKIGYWNQADITCVPKDLLQGEPEVRQGNVTTHGTQEGDVYYVNLKIDGRRINTEVIGTLLDYAITPGDVPWLKKEPLETILK LDLEEQQGTLLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVKPTPKQYHINPKAKPDIQIVINDLLKQGVLIQKESTMNTP VYPVPKPNGRWRMVL DYRAVNKVTP LIAVQNQHSYGILGSLFKGRYKTTIDL SNGFWAHPVPEDYWITAFWQKGQYCWTVLPQGFLNSPGLFN GDVVDLLQGIPNVEVYDDVYISHDSEKEHLEYLDILFNRLKEAGYIISLKKSNANSIVDFLGFQITNEGRGLTDTFKEKLENITAPTTLKQLQSLGLLN FARNFIPDFTELIAPLYALIPKSTKNYPVWQIEHSTTLETITLKNLGAEYLQGRKGDKTILMKVNASYTTGYIRYNEGEKKPISYVSIFSKTELKFTL EKLLTTVHKGLLKALDLSMGQNIHVYSPIVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYPQMPALKDLPVDTGKDNKKHPSNFQHFY TDGSAITSPTKEGHLNAGMGIVYFINKDGNLQKQEWISISLGNHTAQFAEIAAFEFALKKCLPLGGNILLVTDSDNYVAKAYNEELD VWASNGFVNRR KKPLKHISKWKS VADLKRRLRPDVVTHEPGHQKLDSSPHAYGNLADQLATQASFKVH
FFV_O93209_2mut	MDLLKPLTVERKGVKIGYWNQADITCVPKDLLQGEPEVRQGNVTTHGTQEGDVYYVNLKIDGRRINTEVIGTLLDYAITPGDVPWLKKEPLETILK LDLEEQQGTLLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVKPTPKQYHINPKAKPDIQIVINDLLKQGVLIQKESTMNTP VYPVPKPNGRWRMVL DYRAVNKVTP LIAVQNQHSYGILGSLFKGRYKTTIDL SNGFWAHPVPEDYWITAFWQKGQYCWTVLPQGFLNSPGLFN GDVVDLLQGIPNVEVYDDVYISHDSEKEHLEYLDILFNRLKEAGYIISLKKSNANSIVDFLGFQITNEGRGLTDTFKEKLENITAPTTLKQLQSLGLLN FARNFIPDFTELIAPLYALIPKSPKNYPVWQIEHSTTLETITLKNLGAEYLQGRKGDKTILMKVNASYTTGYIRYNEGEKKPISYVSIFSKTELKFTL EKLLTTVHKGLLKALDLSMGQNIHVYSPIVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYPQMPALKDLPVDTGKDNKKHPSNFQHFY TDGSAITSPTKEGHLNAGMGIVYFINKDGNLQKQEWISISLGNHTAQFAEIAAFEFALKKCLPLGGNILLVTDSDNYVAKAYNEELD VWASNGFVNRR KKPLKHISKWKS VADLKRRLRPDVVTHEPGHQKLDSSPHAYGNLADQLATQASFKVH
FFV_O93209_2mutA	MDLLKPLTVERKGVKIGYWNQADITCVPKDLLQGEPEVRQGNVTTHGTQEGDVYYVNLKIDGRRINTEVIGTLLDYAITPGDVPWLKKEPLETILK LDLEEQQGTLLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVKPTPKQYHINPKAKPDIQIVINDLLKQGVLIQKESTMNTP VYPVPKPNGRWRMVL DYRAVNKVTP LIAVQNQHSYGILGSLFKGRYKTTIDL SNGFWAHPVPEDYWITAFWQKGQYCWTVLPQGFLNSPGLFN GDVVDLLQGIPNVEVYDDVYISHDSEKEHLEYLDILFNRLKEAGYIISLKKSNANSIVDFLGFQITNEGRGLTDTFKEKLENITAPTTLKQLQSLGLLN FARNFIPDFTELIAPLYALIPKSPKNYPVWQIEHSTTLETITLKNLGAEYLQGRKGDKTILMKVNASYTTGYIRYNEGEKKPISYVSIFSKTELKFTL EKLLTTVHKGLLKALDLSMGQNIHVYSPIVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYPQMPALKDLPVDTGKDNKKHPSNFQHFY TDGSAITSPTKEGHLNAGMGIVYFINKDGNLQKQEWISISLGNHTAQFAEIAAFEFALKKCLPLGGNILLVTDSDNYVAKAYNEELD VWASNGFVNRR KKPLKHISKWKS VADLKRRLRPDVVTHEPGHQKLDSSPHAYGNLADQLATQASFKVH
FFV_O93209-Proc	VPWLKKEPLETILKLDLEEQQGTLLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVKPTPKQYHINPKAKPDIQIVINDLLKQ GVLIQKES TMNTPVYPVPKPNGRWRMVL DYRAVNKVTP LIAVQNQHSYGILGSLFKGRYKTTIDL SNGFWAHPVPEDYWITAFWQKGQYCWTVLP PQGFINSPLFTGDVVDLLQGIPNVEVYDDVYISHDSEKEHLEYLDILFNRLKEAGYIISLKKSNANSIVDFLGFQITNEGRGLTDTFKEKLENITAPT TLKQLQSLGLLN FARNFIPDFTELIAPLYALIPKSTKNYPVWQIEHSTTLETITLKNLGAEYLQGRKGDKTILMKVNASYTTGYIRYNEGEKKPISYVS IFSKTELKFTLEKLLTTVHKGLLKALDLSMGQNIHVYSPIVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYPQMPALKDLPVDTGKDN KKHPSNFQHFYTDGSAITSPTKEGHLNAGMGIVYFINKDGNLQKQEWISISLGNHTAQFAEIAAFEFALKKCLPLGGNILLVTDSDNYVAKAYNEELD VWASNGFVNRRKKPLKHISKWKS VADLKRRLRPDVVTHEPGHQKLDSSPHAYGNLADQLATQASFKVH
FFV_O93209-	VPWLKKEPLETILKLDLEEQQGTLLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVKPTPKQYHINPKAKPDIQIVINDLLKQ GVLIQKES TMNTPVYPVPKPNGRWRMVL DYRAVNKVTP LIAVQNQHSYGILGSLFKGRYKTTIDL SNGFWAHPVPEDYWITAFWQKGQYCWTVLP PQGFINSPLFTGDVVDLLQGIPNVEVYDDVYISHDSEKEHLEYLDILFNRLKEAGYIISLKKSNANSIVDFLGFQITNEGRGLTDTFKEKLENITAPT

RT Name	RT amino acid sequence
Pro_2mut	TLKQLQSLGKLLNFARNFIPDFTELIAPLYALIPKSPKNYPVWQIEHSTTLETITLKLNGAEYLQGRKGDKTLIMKVNASYTTGYIRYNEGEKKPISYVSI VFSKTELKFTLEKLLTTVHKGLKALDLSMGQNIHVYSPVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYDPQMPALKDLPAVDTGKDN KKHPSNFQHFIFYTDGSAITSPKTEGHLNAGMGIVFYFINKDGNLQKQEWISLGNHTAQFAEIAAEFALKKCLPLGGNLLVTDSDNYVAKAYNEELD VWASNGFVNNRKKPLKHISKWKSADLKRRLPDVVVTHEPGHQKLDSSPHAYGNNADQLATQASFKVH
FFV_0 93209- Pro_2m utA	VPWLKKPLELTIKLDLEEQQGTLNNSILSKKGKEELKQLFEKYSALWQSWENQVGHRRIRPHKIATGTVPKTPQKYHINPKAKPDQIQVINDLLKQ GVLQKESTMTNPVYPVKPNGRWRMVLDYRAVNKVTPLIAVQNQHSYGILGSLFKGRYKTTIDLSNGFWAHPVPEDYWTAFWQKGQYCWTVL PQGFNLNSPGLFNGDVDDLGGIPNVEVYVDDYISHDSEKHELYLDILFNRLKEAGYIISLKKSNIAINSIVDFLGFQITNEGRGLTDTFKEKLENITAPT TLKQLQSLGKLLNFARNFIPDFTELIAPLYALIPKSPKNYPVWQIEHSTTLETITLKLNGAEYLQGRKGDKTLIMKVNASYTTGYIRYNEGEKKPISYVS IVFSKTELKFTLEKLLTTVHKGLKALDLSMGQNIHVYSPVSMQNIQKTPQTAKKALASRWLSWLSYLEDPRIRFFYDPQMPALKDLPAVDTGKDN KKHPSNFQHFIFYTDGSAITSPKTEGHLNAGMGIVFYFINKDGNLQKQEWISLGNHTAQFAEIAAEFALKKCLPLGGNLLVTDSDNYVAKAYNEELD VWASNGFVNNRKKPLKHISKWKSADLKRRLPDVVVTHEPGHQKLDSSPHAYGNNADQLATQASFKVH
FLV_P1 0273	TLQLEEEYRLFEPSTQKQEMDIWLNKFPQAWAETGGMGTAHCQAPVLQIKATATPISIRQYMPHEAYQGKIPHIRMLDQGLKPCQSPWNT LLPVKKPGTEDYRPVQDLREVNKRVEDIHPTVPNPYNLLSTLPPSHPWYTVLDLDAFFCLRLHSESQLLFAFEWRDPEIGLGGQLTWTRLPGQFK NSPTLFDEALHSDADFVRYPALVLLQYVDDLAAATRTTECLEGTKALLETGNKGYRASAKKAQICLQEVTVLGYSLKDGQRWLTAKARAILSI PVPKNSRQVREFLGAGYCRWLWPGFAELAAPLYLTPRGTFLQWGTEQQLAFEDIKALLSSPALGLPDITKPFELFIDENSFGAKGVLVQKLGW KRPVAYLSKKLDTVASGWPPCLRMVAIAAILVKDAGKLTGGPLTILTSHPEALVRQPPNKLWLNARMTHYQAMLLDAERVHFGPTVSLNPATLLP LPSGGNHHDCLQILAETHGTRPDLTDQPLPADLTWYTDGSSFIRNGERECAAVTTESEVIWAAPLPPGTSQAQRAELIALTQALKMAEGKKLT DSRYAFATTHVHGEYRRRGWLTSERGEIKNKNEILALLEALFLPKRLSIHCPGHQKGDSPQAKGNRLADDTAKKAATETHSSLTVLPL
FLV_P1 0273_3 mut	TLQLEEEYRLFEPSTQKQEMDIWLNKFPQAWAETGGMGTAHCQAPVLQIKATATPISIRQYMPHEAYQGKIPHIRMLDQGLKPCQSPWNT LLPVKKPGTEDYRPVQDLREVNKRVEDIHPTVPNPYNLLSTLPPSHPWYTVLDLDAFFCLRLHSESQLLFAFEWRDPEIGLGGQLTWTRLPGQFK NSPTLFDEALHSDADFVRYPALVLLQYVDDLAAATRTTECLEGTKALLETGNKGYRASAKKAQICLQEVTVLGYSLKDGQRWLTAKARAILSI PVPKNSRQVREFLGAGYCRWLWPGFAELAAPLYLTPRGTFLQWGTEQQLAFEDIKALLSSPALGLPDITKPFELFIDENSFGAKGVLVQKLGW KRPVAYLSKKLDTVASGWPPCLRMVAIAAILVKDAGKLTGGPLTILTSHPEALVRQPPNKLWLNARMTHYQAMLLDAERVHFGPTVSLNPATLLP LPSGGNHHDCLQILAETHGTRPDLTDQPLPADLTWYTDGSSFIRNGERECAAVTTESEVIWAAPLPPGTSQAQRAELIALTQALKMAEGKKLT DSRYAFATTHVHGEYRRRGWLTSERGEIKNKNEILALLEALFLPKRLSIHCPGHQKGDSPQAKGNRLADDTAKKAATETHSSLTVLPL
FLV_P1 0273_3 mutA	TLQLEEEYRLFEPSTQKQEMDIWLNKFPQAWAETGGMGTAHCQAPVLQIKATATPISIRQYMPHEAYQGKIPHIRMLDQGLKPCQSPWNT LLPVKKPGTEDYRPVQDLREVNKRVEDIHPTVPNPYNLLSTLPPSHPWYTVLDLDAFFCLRLHSESQLLFAFEWRDPEIGLGGQLTWTRLPGQFK NSPTLFDEALHSDADFVRYPALVLLQYVDDLAAATRTTECLEGTKALLETGNKGYRASAKKAQICLQEVTVLGYSLKDGQRWLTAKARAILSI PVPKNSRQVREFLGAGYCRWLWPGFAELAAPLYLTPRGTFLQWGTEQQLAFEDIKALLSSPALGLPDITKPFELFIDENSFGAKGVLVQKLGW KRPVAYLSKKLDTVASGWPPCLRMVAIAAILVKDAGKLTGGPLTILTSHPEALVRQPPNKLWLNARMTHYQAMLLDAERVHFGPTVSLNPATLLP LPSGGNHHDCLQILAETHGTRPDLTDQPLPADLTWYTDGSSFIRNGERECAAVTTESEVIWAAPLPPGTSQAQRAELIALTQALKMAEGKKLT DSRYAFATTHVHGEYRRRGWLTSERGEIKNKNEILALLEALFLPKRLSIHCPGHQKGDSPQAKGNRLADDTAKKAATETHSSLTVLPL
FOAMV _P1435 0	MNPLQLLQPLPAEIKGTLLAHWNNSGATITCIPESFLEDEQPIKKTUKTIHGEKQNVVYVTFKVKGRKVEAEVIAASPYEYILLSPDTPVWL TQQPLQLTILVPLQEYQEILSKTALPEDQKQQLKTLFVKYDNLWQHWNQVGHKIRPHNIATGDYPPRPQKQYIPNPKAKPSIQMIDLLKQGVLPQNSTM NTPVYPVPKPDGRWRMVLDYREVNTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQKGQYCWTRLPGQFLNSPA LFNADVDLLKEIPNVQVYVDDIYLSHDDPKEHVQKLEKVFQILLQAGYVVSLLKKEIGQKTVEFLGFNITKEGRGLTDTFTKLLNITPPKDLKQLQSI LGLLNFARNFIPNFAELVQPLYNLIASAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIKVNTPSPAGYVRYNETGKKPIMLYNVFSKA ELKFSMLEKLLTTMHKALIKAMDAMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELKHIPDVYTSQSPVKHPSQ YEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWSIPLGNHTAQMAEIAAEVAFACKKALKIPGPVLVITDSFYVAESANKELPYWKSNG FVNNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYYVN
FOAMV _P1435 0_2mut	MNPLQLLQPLPAEIKGTLLAHWNNSGATITCIPESFLEDEQPIKKTUKTIHGEKQNVVYVTFKVKGRKVEAEVIAASPYEYILLSPDTPVWL TQQPLQLTILVPLQEYQEILSKTALPEDQKQQLKTLFVKYDNLWQHWNQVGHKIRPHNIATGDYPPRPQKQYIPNPKAKPSIQMIDLLKQGVLPQNSTM NTPVYPVPKPDGRWRMVLDYREVNTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQKGQYCWTRLPGQFLNSPA LFNADVDLLKEIPNVQVYVDDIYLSHDDPKEHVQKLEKVFQILLQAGYVVSLLKKEIGQKTVEFLGFNITKEGRGLTDTFTKLLNITPPKDLKQLQSI LGLLNFARNFIPNFAELVQPLYNLIAPAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIKVNTPSPAGYVRYNETGKKPIMLYNVFSKA ELKFSMLEKLLTTMHKALIKAMDAMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELKHIPDVYTSQSPVKHPSQ YEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWSIPLGNHTAQMAEIAAEVAFACKKALKIPGPVLVITDSFYVAESANKELPYWKSNG FVNNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYYVN
FOAMV _P1435 0_2mut A	MNPLQLLQPLPAEIKGTLLAHWNNSGATITCIPESFLEDEQPIKKTUKTIHGEKQNVVYVTFKVKGRKVEAEVIAASPYEYILLSPDTPVWL TQQPLQLTILVPLQEYQEILSKTALPEDQKQQLKTLFVKYDNLWQHWNQVGHKIRPHNIATGDYPPRPQKQYIPNPKAKPSIQMIDLLKQGVLPQNSTM NTPVYPVPKPDGRWRMVLDYREVNTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQKGQYCWTRLPGQFLNSPA LFNADVDLLKEIPNVQVYVDDIYLSHDDPKEHVQKLEKVFQILLQAGYVVSLLKKEIGQKTVEFLGFNITKEGRGLTDTFTKLLNITPPKDLKQLQSI LGLLNFARNFIPNFAELVQPLYNLIAPAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIKVNTPSPAGYVRYNETGKKPIMLYNVFSKA ELKFSMLEKLLTTMHKALIKAMDAMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELKHIPDVYTSQSPVKHPSQ YEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWSIPLGNHTAQMAEIAAEVAFACKKALKIPGPVLVITDSFYVAESANKELPYWKSNG FVNNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYYVN
FOAMV _P1435 0-Pro	VPWLTTQQLQLTILVPLQEYQEILSKTALPEDQKQQLKTLFVKYDNLWQHWNQVGHKIRPHNIATGDYPPRPQKQYIPNPKAKPSIQMIDLLK QGVLPQNSTMTNPVYPVPKPDGRWRMVLDYREVNTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQKGQYCW RLPQGFNLNSPALFNADVDLLKEIPNVQVYVDDIYLSHDDPKEHVQKLEKVFQILLQAGYVVSLLKKEIGQKTVEFLGFNITKEGRGLTDTFTKLLNI TPPKDLKQLQSLGKLLNFARNFIPNFAELVQPLYNLIASAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIKVNTPSPAGYVRYNETGKK PIMLYNVFSKAELKFSMLEKLLTTMHKALIKAMDAMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELKHIPDVY TSQSPVKHPSQYEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWSIPLGNHTAQMAEIAAEVAFACKKALKIPGPVLVITDSFYVAESA NKELPYWKSNGFVNNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYYVN
FOAMV _P1435 0-	VPWLTTQQLQLTILVPLQEYQEILSKTALPEDQKQQLKTLFVKYDNLWQHWNQVGHKIRPHNIATGDYPPRPQKQYIPNPKAKPSIQMIDLLK QGVLPQNSTMTNPVYPVPKPDGRWRMVLDYREVNTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQKGQYCW RLPQGFNLNSPALFNADVDLLKEIPNVQVYVDDIYLSHDDPKEHVQKLEKVFQILLQAGYVVSLLKKEIGQKTVEFLGFNITKEGRGLTDTFTKLLNI TPPKDLKQLQSLGKLLNFARNFIPNFAELVQPLYNLIAPAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIKVNTPSPAGYVRYNETGKK

RT Name	RT amino acid sequence
Pro_2mut	PIMYLNVFSKAEKFSMLEKLLTTHKALIKAMDAMGQELVSPVISMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPELKHIPDVYTSSQSPVKHPSQYEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWISPLGNHTAQMAEIAAEVFAACKKALKIPGVLVITDSFYVAESA NKELPYWKSNGFVNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYVNN
FOAMV_P1435 0- Pro_2mutA	VPWLQTQQLQLTILVPLQEYQEKILSKTALPEDQKQKLTLFVKYDNLWQHWHENQVGHKIRPHNIATGDYPPRPQKQYPINPKAKPSIQIVDDLKQGVLTQPNSTMTNTPVYPVKPDGRWRMVL DYREVNKTIPLTAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPESYWLTAFTWQGGKQYCWTRLPQGGLNSPALFNADVVDLLKEIPNVQVYVDDIYSHDDPKHEVQQLKVFQILLQAGYVVSLLKSEIGQKTVFELGFINTEKGRGLTDTFKTKLLNI TPPKDLKQLQKILGKLNFAFNFIPIFAELVQPLYNLIAPAKGKYIEWSEENTKQLNMVIEALNTASNLEERLPEQRLVIVKNTSPSAGYVRYNETGKK PIMYLNVFSKAEKFSMLEKLLTTHKALIKAMDAMGQELVSPVISMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPELKHIPDVYTSSQSPVKHPSQYEGVFYTDGSAIKSPDPTKSNAGMGIVHATYKPEYQVLNQWISPLGNHTAQMAEIAAEVFAACKKALKIPGVLVITDSFYVAESA NKELPYWKSNGFVNKKKPLKHISKWKSIAECLSMKPDITIQHEKGISLQIPVILKGNALADKLATQGSYVNN
GALV_P21414	VLNLEEEYRLHEKVPSSIDPSWLQLFPTVWAERAGMCLANQVPPVVELRSGASPVAVRQYPMSEAREGIRPHIQKFLDLGVLVPCRSWNTPLLPVKKPGTNDYRPVQDLREINKRVQDIHPTVPNPYNLLSSLPSTYTWYSVLDLKDFAFFCLRLHPNSQPLFAFEWKDPEKNGTQGLTWTRLPQGFKNSTPLFDEALHRDLAPFRALNPQVLLQYVDDLLVAAPTYEDCKGTQKLLQELSKLGYRVSAKKAQLCQREVITYLGYLLKEGKRWLTPARKATVM KIPVPTTPRQVREFLGTAGFCRLWIPGFASLAAPLYPLTKESIPFIWTEEHQAFDHIKKALLSAPALALPDLTKPFTLYIDERAGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKDADKLTGQNVTVIASHLESIVRQPPDRWMTNARMTHYSLLNERVSFAPPVLPATLLPV ESEATPVHRCSELAEEETGTRRDLEDQPLPGVPTWYTDGSSFTTEGKRRAGAPIVDGKRTVWASSLPEGTSQAQKAEVALTQALRLAEGKNINIYD SRYAFATAIHGAIYKQRGWLTSAQKDIKNKEEILALLEIHLPRRVAIHCPGHQGRGNSNPVATGNRRADEAAKQAALSTRVLAGTTKP
GALV_P21414_3mut	VLNLEEEYRLHEKVPSSIDPSWLQLFPTVWAERAGMCLANQVPPVVELRSGASPVAVRQYPMSEAREGIRPHIQKFLDLGVLVPCRSWNTPLLPVKKPGTNDYRPVQDLREINKRVQDIHPTVPNPYNLLSSLPSTYTWYSVLDLKDFAFFCLRLHPNSQPLFAFEWKDPEKNGTQGLTWTRLPQGFKNSTPLFNEALHRDLAPFRALNPQVLLQYVDDLLVAAPTYEDCKGTQKLLQELSKLGYRVSAKKAQLCQREVITYLGYLLKEGKRWLTPARKATVM KIPVPTTPRQVREFLGTAGFCRLWIPGFASLAAPLYPLTKPSIPFIWTEEHQAFDHIKKALLSAPALALPDLTKPFTLYIDERAGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKDADKLTGQNVTVIASHLESIVRQPPDRWMTNARMTHYSLLNERVSFAPPVLPATLLPV ESEATPVHRCSELAEEETGTRRDLEDQPLPGVPTWYTDGSSFTTEGKRRAGAPIVDGKRTVWASSLPEGTSQAQKAEVALTQALRLAEGKNINIYD SRYAFATAIHGAIYKQRGWLTSAQKDIKNKEEILALLEIHLPRRVAIHCPGHQGRGNSNPVATGNRRADEAAKQAALSTRVLAGTTKP
GALV_P21414_3mutA	VLNLEEEYRLHEKVPSSIDPSWLQLFPTVWAERAGMCLANQVPPVVELRSGASPVAVRQYPMSEAREGIRPHIQKFLDLGVLVPCRSWNTPLLPVKKPGTNDYRPVQDLREINKRVQDIHPTVPNPYNLLSSLPSTYTWYSVLDLKDFAFFCLRLHPNSQPLFAFEWKDPEKNGTQGLTWTRLPQGFKNSTPLFNEALHRDLAPFRALNPQVLLQYVDDLLVAAPTYEDCKGTQKLLQELSKLGYRVSAKKAQLCQREVITYLGYLLKEGKRWLTPARKATVM KIPVPTTPRQVREFLGTAGFCRLWIPGFASLAAPLYPLTKPSIPFIWTEEHQAFDHIKKALLSAPALALPDLTKPFTLYIDERAGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKDADKLTGQNVTVIASHLESIVRQPPDRWMTNARMTHYSLLNERVSFAPPVLPATLLPV ESEATPVHRCSELAEEETGTRRDLEDQPLPGVPTWYTDGSSFTTEGKRRAGAPIVDGKRTVWASSLPEGTSQAQKAEVALTQALRLAEGKNINIYD SRYAFATAIHGAIYKQRGWLTSAQKDIKNKEEILALLEIHLPRRVAIHCPGHQGRGNSNPVATGNRRADEAAKQAALSTRVLAGTTKP
HTL1A_P0336 2	AVLGLEHLPRPPQISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFEMQLAHILQPIRQAFPPQCTILQYMDILLASPSHEDLLLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSRAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLSRKVYVYHHVRSHTNLPDPISRLNALTDALLITPVLQ
HTL1A_P0336 2_2mut	AVLGLEHLPRPPQISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFQMQLAHILQPIRQAFPPQCTILQYMDILLASPSHEDLLLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSRAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLSRKVYVYHHVRSHTNLPDPISRLNALTDALLITPVLQ
HTL1A_P0336 2_2mut B	AVLGLEHLPRPPQISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFQMQLAHILQPIRQAFPPQCTILQYMDILLASPSHEDLLLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSRAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLSRKVYVYHHVRSHTNLPDPISRLNALTDALLITPVLQ
HTL1C_P1407 8	AVLGLEHLPRPPEISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFEMQLAHILQPIRQAFPPQCTILQYMDILLASPSHADLQLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSQAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLSRKVYVYHHVRSHTNLPDPISRLNALTDALLITPVLQ
HTL1C_P1407 8_2mut	AVLGLEHLPRPPEISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFQMQLAHILQPIRQAFPPQCTILQYMDILLASPSHADLQLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSQAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLSRKVYVYHHVRSHTNLPDPISRLNALTDALLITPVLQ
HTL1L_P0C211	GLEHLPRPPEISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTIDLSSSSPGPPDLSSLPPTLAHLQ TIDLDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPPQGFKNSTPLFEMQLASILQPIRQAFPPQCTILQYMDILLASPSPEDLQLSEATMASLISHGLPVSENKTQQTPTGKIFLQGIISPNHLTYDAVPTVPIRSRWALPELQALLGEIQWVSKGTPTLRQPLHSLYCALQPHTDPRDQIYNPSQV QSLVQLRQALSQNCRSRLVQTLPLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQGQLLASAVLLDKYTLQSYGLLCQTIHNNISTQT FNQFIQTSDHPSVPIILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALMPVFTLSPVIINTAPCLFSDGSTSQAAYILWDKILSQRSFPLPPPHKS AQRAELLGLLHGLSSARSWRCLNIFLDSKYLYHYLRTLALGTGQRSSQAPFQALLPRLLAHKVYVYHHVRSHTNLPDPISRLNALTDALLITPIL

RT Name	RT amino acid sequence
HTL1L_P0C211_2mut	GLEHLPRPPEISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTVDLSSSSPGPPDLSSLPPTLAHLQTDILKDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPGGFKNSPTLFQMQLASILQPIRQAFPPQCVILQYMDILLASPSPEDLQQLSEATMASLISHGLPVSDQDKTQQTGKIFLGGIISPNHITYDAVPTVPIRSRWALPELQALLGEIQWWSKGTPLRQPLHSLYCALQGHOTDPRDQIYNPSQVQSLMQLQQALSQNCRSRLAQTLLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQQLASAVLLDKYTLQSYGLLCQTHHNSIQTFNQFIQTSDHPSVPILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALTPVFTLSPIINTAPCLFSDGSGTAAAYILWDKHLQRSFPLPPPHKSAQQAELLGLLHGLSSARSWHCLNIFLDSKYLHYLRTLAWGTFQGGSSQAPFQALLPRLLAHKVIYLVHVRSHNTLNDPISRLNEATDALMLAPLL
HTL1L_P0C211_2mutB	GLEHLPRPPEISQFPLNPERLQALQHLVRKALEAGHIEPYTGPNNPVFPVKKANGTWRFIHDLRATNSLTVDLSSSSPGPPDLSSLPPTLAHLQTDILKDAFFQIPLPKQFQPYFAFTVPQQCNYPGTRYAWKVLPGGFKNSPTLFQMQLASILQPIRQAFPPQCVILQYMDILLASPSPEDLQQLSEATMASLISHGLPVSDQDKTQQTGKIFLGGIISPNHITYDAVPTVPIRSRWALPELQALLGEIQWWSKGTPLRQPLHSLYCALQGHOTDPRDQIYNPSQVQSLMQLQQALSQNCRSRLAQTLLGAIMLTLTGTTTVFQSKQWPLVWLHAPLPHTSQCPWQQLASAVLLDKYTLQSYGLLCQTHHNSIQTFNQFIQTSDHPSVPILLHSHRFKNLGAQTGELWNTFLKTAAPLAPVKALTPVFTLSPIINTAPCLFSDGSGTAAAYILWDKHLQRSFPLPPPHKSAQQAELLGLLHGLSSARSWHCLNIFLDSKYLHYLRTLAWGTFQGGSSQAPFQALLPRLLAHKVIYLVHVRSHNTLNDPISRLNEATDALMLAPLL
HTL32_Q0R5R2	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSVTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPTIFQPYFAFTLPQNNYGPTRYSWRVLPGGFKNSPTLFQQLSHILTPVRKTFPNSLIQYMDILLASPAGEAALTDKVTNALTKEGPLPSPEKTQATPGPIHFLGQVISQDCITYETLPSINVKSTWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIKLTSIQVQALRTIQKALTINCRSRLVNQLPILALIMLRPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVINHAPCLFSDGASAKAAFIWDRQVIHQVQLSLPSTCSAQAGELFGLLAGLQKSPQWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCELTHTQLLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTL32_Q0R5R2_2mut	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSVTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPTIFQPYFAFTLPQNNYGPTRYSWRVLPGGFKNSPTLFQQLSHILTPVRKTFPNSLIQYMDILLASPAGEAALTDKVTNALTKEGPLPSPEKTQATPGPIHFLGQVISQDCITYETLPSINVKSTWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIKLTSIQVQALRTIQKALTINCRSRLVNQLPILALIMLRPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVINHAPCLFSDGASAKAAFIWDRQVIHQVQLSLPSTCSAQAGELFGLLAGLQKSPQWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCELTHTQLLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTL32_Q0R5R2_2mutB	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSVTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPTIFQPYFAFTLPQNNYGPTRYSWRVLPGGFKNSPTLFQQLSHILTPVRKTFPNSLIQYMDILLASPAGEAALTDKVTNALTKEGPLPSPEKTQATPGPIHFLGQVISQDCITYETLPSINVKSTWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIKLTSIQVQALRTIQKALTINCRSRLVNQLPILALIMLRPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVINHAPCLFSDGASAKAAFIWDRQVIHQVQLSLPSTCSAQAGELFGLLAGLQKSPQWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCELTHTQLLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTL3P_Q4U0X6	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSLTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPAVFQPYFAFTLPQNNHGPTRYSWRVLPGGFKNSPTLFQQLSHILAPVRKAFPNLSLIQYMDILLASPALRELTALTDKVTNALTKEGPLMSLEKTQATPGSIHFLGQVISQDCITYETLPSIHVKSISWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIELTSTQVQALKTIQKALANCRSRLVSQPLALILRLPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVIDHAPCLFSDGATSKAAFIWDRQVIHQVQLPLPSTCSAQAGELFGLLAGLQKSPWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCDLHARLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTL3P_Q4U0X6_2mut	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSLTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPAVFQPYFAFTLPQNNHGPTRYSWRVLPGGFKNSPTLFQQLSHILAPVRKAFPNLSLIQYMDILLASPALRELTALTDKVTNALTKEGPLMSLEKTQATPGSIHFLGQVISQDCITYETLPSIHVKSISWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIELTSTQVQALKTIQKALANCRSRLVSQPLALILRLPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVIDHAPCLFSDGATSKAAFIWDRQVIHQVQLPLPSTCSAQAGELFGLLAGLQKSPWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCDLHARLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTL3P_Q4U0X6_2mutB	GLEHLPPPPEVSQFPLNPERLQALTDLSRALEAKHIEPYQGPGNNPIFPVKKPNGKWRFIHDLRATNSLTRDLASPSGPPDLTSLPQGLPHLRTILDTDAFFQIPLPAVFQPYFAFTLPQNNHGPTRYSWRVLPGGFKNSPTLFQQLSHILAPVRKAFPNLSLIQYMDILLASPALRELTALTDKVTNALTKEGPLMSLEKTQATPGSIHFLGQVISQDCITYETLPSIHVKSISWSLAELQSMGELQWWSKGTPLVRSSHLQYLALRGHRDPRDTIELTSTQVQALKTIQKALANCRSRLVSQPLALILRLPTGTTAVLFQTKQKWPLVWLHTPHPATSLRPWGQLANAVILDKYSLQHYGQVCKSFHHNISNQAALTYYLHTSDQSSVAILLQSHSRFHNLAGQPSGPWRSLLQMPQIFQNIQVLRPPFTISPVIDHAPCLFSDGATSKAAFIWDRQVIHQVQLPLPSTCSAQAGELFGLLAGLQKSPWVALNIFLDSKFLIGHLRMAWGAFFGPSTQCDLHARLPLQGGKTVYVHVRSHTLLQDPISRLNEATDALMLAPLL
HTLV2_P03363_2mut	HLPPPPQVQDFPLNPERLQALNDLSKALEAGHIEPYSGPGNNPVFPVKKPNGKWRFIHDLRATNAITTLTSPSPGPPDLTSLPTALPHLQTDILTDAFFQIPLPKQYQPYFAFTIPQPCNYGPGTRYAWTVLPQGFKNSPTLFQQLAAVLNPMRMKMFPTSTIVQYMDILLASPTNEELQQLSQLTLQALTTHGLPISQEKTKQTGQIRFLGQVISPNHITYESTPTIPIKSQWTLTELQVILGEIQWWSKGTPLRKHLQSLYSALHPYRDRPRACITLTPQQLHALHAIQQAALQHNCRGRNLNPLALLGLISLSTSGTTSVIFQPKQNWPLAWLHTHPPTSLCPWGHLLACTILTLDKYTLQHYGQLCQSFHHNMSKQALCDFLRNSPHSPVGLIIHMGGRFHNLSQSPSGPWKTLHLPTLLQEPRLLRPIFTLSPVVLDTAPCLFSDGSPQKAAAYLVWDQITLQDITPLPSHETHSAQKGLLELLALICGLRAAKWPVALNIFLDSKYLKYLHSLAIGALGTSAHQTLQAALPPLQGGKTYLVHVRSHNTLNDPISFTNEYDLSLAPLVPL
JSRV_P31623	PLGTSDFVTHADPIDWKSSEEPVWVWQWPLTQEKLAAQQLVQEQRLGHIEPSTSAWNSPIFVKKKSGKWRLLQDLRKVNETMMHMGALQPLPTPSAIFDKSYIIVIDLKDCFYTIAPQDCKRFAFSLPSVNFKEPMQRYQWRVLPGQMTNSPTLCQKFVATAIAPVRQRFQQLVHYHMDILLAHTDHLLYQAFSILKQHLNLGLVIADEKIQTHFPYNYLGFSLYPRVYNTQLVKLQTDHLKTLNDFQKLLGDINWIRPYLKLPTYTLQPLFDILKGDSDPASPRTLSEGRALQSIEAIRQQQITYCDYQRSWGLYILTPRAPTGVLQDKPLRWIYLSATPTKHLPPYELVAKIAKGRHEAIQYFGMEPPFICVPYALEQQDWLFQFSDNWSIAFANYPGQITHYPSDKLLQFASSHAIFPKIVRRQPIPEATLIFTDSSNGTAALINHQTYYAQTSFSSAQWELFAVHQALLTVPTSFNLTDSYVVGALQMIEVTPIIGTTSPEVLNFTLIQQVLLHCRQHPCFFGHIRAHSTLPGALVQGNHTADVLTKQVFFQS
JSRV_P31623_2mutB	PLGTSDFVTHADPIDWKSSEEPVWVWQWPLTQEKLAAQQLVQEQRLGHIEPSTSAWNSPIFVKKKSGKWRLLQDLRKVNETMMHMGALQPLPTPSPIFDKSYIIVIDLKDCFYTIAPQDCKRFAFSLPSVNFKEPMQRYQWRVLPGQMTNSPTLCQKFVATAIAPVRQRFQQLVHYHMDILLAHTDHLLYQAFSILKQHLNLGLVIADEKIQTHFPYNYLGFSLYPRVYNTQLVKLQTDHLKTLNDFQKLLGDINWIRPYLKLPTYTLQPLFDILKGDSDPASPRTLSEGRALQSIEAIRQQQITYCDYQRSWGLYILTPRAPTGVLQDKPLRWIYLSATPTKHLPPYELVAKIAKGRHEAIQYFGMEPPFICVPYALEQQDWLFQFSDNWSIAFANYPGQITHYPSDKLLQFASSHAIFPKIVRRQPIPEATLIFTDSSNGTAALINHQTYYAQTSFSSAQWELFAVHQALLTVPTSFNLTDSYVVGALQMIEVTPIIGTTSPEVLNFTLIQQVLLHCRQHPCFFGHIRAHSTLPGALVQGNHTADVLTKQVFFQS

RT Name	RT amino acid sequence
KORV_Q9TTC 1	TLGDQGSRGSDPLPEPRVLTVEGIPTFELVNTGAEHSVLTKPMGKMGSKRTVAGATGSKVYPWTTKRLLKIGQKQVTHSFLVIPECAPILLGRD LLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQYPMSE AREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLHPNSQP LFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFDEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVSACKAQL CREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLSAPALA LPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDRWMTNA RMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVWASNL EGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATGNRKAD EAAKQAAQSTRILTETTKN
KORV_Q9TTC 1_3mut	TLGDQGSRGSDPLPEPRVLTVEGIPTFELVNTGAEHSVLTKPMGKMGSKRTVAGATGSKVYPWTTKRLLKIGQKQVTHSFLVIPECAPILLGRD LLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQYPMSE AREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLHPNSQP LFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFNEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVSACKAQL CREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLSAPALA LPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDRWMTNA RMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVWASNL EGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATGNRKA DEAAKQAAQSTRILTETTKN
KORV_Q9TTC 1_3mut A	TLGDQGSRGSDPLPEPRVLTVEGIPTFELVNTGAEHSVLTKPMGKMGSKRTVAGATGSKVYPWTTKRLLKIGQKQVTHSFLVIPECAPILLGRD LLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQYPMSE AREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLHPNSQP LFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFNEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVSACKAQL CREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLSAPALA LPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDRWMTNA RMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVWASNL EGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATGNRKA DEAAKQAAQSTRILTETTKN
KORV_Q9TTC 1-Pro	LLGRDLLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQY PMSKEAREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLH PNSQPLFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFDEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVS AKAQLCREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLS APALALPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDR WMTNARMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVW ASNLPEGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATG NRKADEAAKQAAQSTRILTETTKN
KORV_Q9TTC 1-Pro_3mut	LLGRDLLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQY PMSKEAREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLH PNSQPLFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFNEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVS AKAQLCREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLS APALALPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDR WMTNARMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVW ASNLPEGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATG NRKADEAAKQAAQSTRILTETTKN
KORV_Q9TTC 1-Pro_3mut A	LLGRDLLTKLKAQIQFSTEGPQVTWEDRPAMCLVLNLEEEYRLHEKPVPSIDPSWLQLFPMVWAEKAGMGLANQVPPVVELKSDASPAVAVRQY PMSKEAREGIRPHIQRFLLDLGILVPCQSPWNTPLLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSSLPPSHTWYSVLDLKDAFFCLKLH PNSQPLFAFEWRDPEKNGTQGLTWTRLPQGFKNSPTLFNEALHRDLASFALNPQVVMQLQYVDDLVAAPTYRDCKEGTRRLQELSKLGYRVS AKAQLCREEVTYLYGILLKGGKRWLTPARKATVMKIPTPTTPRQVREFLGAGFCRLWIPGFASLAAPLYPLTRPKVPFTWTEAHQEAFGRIKEALLS APALALPDLTKPFALYVDEKEGVARGVLTQTLGPWRRPVAYLSKKLDPVAGSWPTCLKAIAAVALLKDADKLTGQNVLIAPHNLESIVRQPPDR WMTNARMTHYQSLLLNERVSFAPPAILNPATLLPVESDDTPIHCSEILAETGTRPDLRDQPLPGVPAWYTDGSSFIMDGRGAGAAIVDNKRTVW ASNLPEGTSAQKAEIALTQALRLAEGKSINIYDSRYAFATAHVHGAIFYKQRGWLTSGAKDIKNKEELALLEIAHLPKRVAIHCPCGHQRGTDVPVATG NRKADEAAKQAAQSTRILTETTKN
MLVAV_P0335 6	TLNLEDEYRLYETSAEPEVSPGTSWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSQKQYPMSEAKLGKPHIQRLLDQGILVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSGLPPSHRWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPMGSGSQGLTWTRLPQGF KNSPTLFDEALHRDLADFRIQHPDLILLQYVDDILLATSELDCQGGTRALLTLGNLGYRASAKKAQLCQKQVKYLYGILLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGAGFCRLWIPGFASLAAPLYPLTKGTFLNFWGPDQKQAYQEIQALLTAPALGLPDLTKPFELFVDEKQYAKGVLTKQL GPWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLKDKAGLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQGFVVALN PATLLPLEEGAPHDCLEILAETHGTRPDLTDQIPDADHTWYTDGSSFLQEGQRKAGAAVTETETVIWARALPAGTSAQRAELIALTQALKMAEGK RLNVYTDSDRYAFATAHIHGEIYRRRGWLTSEGREIKNKSEILALLKALFLPKRLSIHCLGHQKGDSEARGNRLADQAAAREAAIKTPPDTSTLL
MLVAV_P0335 6_3mut	TLNLEDEYRLYETSAEPEVSPGTSWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSQKQYPMSEAKLGKPHIQRLLDQGILVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSGLPPSHRWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPMGSGSQGLTWTRLPQGF KNSPTLFNEALHRDLADFRIQHPDLILLQYVDDILLATSELDCQGGTRALLTLGNLGYRASAKKAQLCQKQVKYLYGILLKEGQRWLTEARKETVM GQPTPKTPRQLREFLGAGFCRLWIPGFASLAAPLYPLTKGTFLNFWGPDQKQAYQEIQALLTAPALGLPDLTKPFELFVDEKQYAKGVLTKQL GPWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLKDKAGLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQGFVVALN PATLLPLEEGAPHDCLEILAETHGTRPDLTDQIPDADHTWYTDGSSFLQEGQRKAGAAVTETETVIWARALPAGTSAQRAELIALTQALKMAEGK RLNVYTDSDRYAFATAHIHGEIYRRRGWLTSEGREIKNKSEILALLKALFLPKRLSIHCLGHQKGDSEARGNRLADQAAAREAAIKTPPDTSTLL
MLVAV_P0335	TLNLEDEYRLYETSAEPEVSPGTSWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSQKQYPMSEAKLGKPHIQRLLDQGILVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREVNRVQDIHPTVPNPYNLLSGLPPSHRWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPMGSGSQGLTWTRLPQGF

[illegible]

RT Name	RT amino acid sequence
	LLPLPEEGLQHDCLDILAEAHGTRSDLMQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVF5 _P2681 0	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAFRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWKDPPEMGISGQLTWTRLPGQFK NSPTLFDEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFKWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDVGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPAT LLPLPEEGLQHDCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVF5 _P2681 0_3mut	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAFRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWKDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFKWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDVGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPAT LLPLPEEGLQHDCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVF5 _P2681 0_3mut A	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAFRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWKDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFKWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDVGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPAT LPLPEEGLQHDCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVFF _P2680 9_3mut	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFEWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPAT LLPLPEEGLQHDCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVFF _P2680 9_3mut A	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFEWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPAT LPLPEEGLQHDCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSFLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKLN VYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAAREVATRETPESTLL
MLVMS _P0335 5	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFDEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFNWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPA TLPLPEEGLQHNCILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAARKAAITETPDSTLL
MLVMS _P0335 5	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFDEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFNWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPA TLPLPEEGLQHNCILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAARKAAITETPDSTLL
MLVMS _P0335 5_3mut	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFNWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPA TLPLPEEGLQHNCILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAARKAAITETPDSTLL
MLVMS _P0335 5_3mut	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLALLAATSELDCQQGTRALLQTLGDLGYRASAKKAKICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGTAGLCRLWIPGFAEMAAPLYPLTKGTGLFNWGPDDQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAAGWPPCLRMVAIAVLTAKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDRVQFGPIVALNPA TLPLPEEGLQHNCILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTTETETEVWAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSDRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGNHAEARGNRMADQAARKAAITETPDSTLL
MLVMS _P0335	TLNIEDEYRLHETSKGPDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIISLKATSTPVSIKQYPMSEARLGKPHIQRLLDQGILVPCQSPWNTPL LPVKKPGTNDYRVPQDLREVNRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRHLHPTSQSLFAFEWRDPPEMGISGQLTWTRLPGQFK

RT Name	RT amino acid sequence
5_3mut A_WS	NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDDQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLDTRVQFGPVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLL
MLVMS _P0335 5_3mut A_WS	TLNIEDEHRLHETSKPEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIIKQYPMSEARLGKIPHIQRLDQGLVPCQSPWNTPL LPVKKPGTNDYRVPVQDLREVNRVEDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPGEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDDQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLDTRVQFGPVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLL
MLVMS _P0335 5_PLV9 19	TLNIEDEYRLHETSKPEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIIKQYPMSEARLGKIPHIQRLDQGLVPCQSPWNTPL LPVKKPGTNDYRVPVQDLREVNRVEDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPGEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDDQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLDTRVQFGPVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP SGGSKRTADGSEFE
MLVMS _P0335 5_PLV9 19	TLNIEDEYRLHETSKPEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIIKQYPMSEARLGKIPHIQRLDQGLVPCQSPWNTPL LPVKKPGTNDYRVPVQDLREVNRVEDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPGEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLKEGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDDQKAYQEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLDTRVQFGPVALNPA TLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSLLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEARGNRMADQAARKAAITETPDTSTLLIENSSP SGGSKRTADGSEFE
MLVRD _P1122 7	TLNIEDEYRLHEISTEPDVSPGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIIKQYPMSEARLGKIPHIQRLDQGLVPCQSPWNTPL LPVKKPGTNDYRVPVQDLREVNRVEDIHPTVNPYNLLSGLPTSHRWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPGEMGISGQLTWTRLPQGFK NSPTLFDEALHRGLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLREGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLWIPRFAEMAAPLYPLTKPGTLFNWGPDDQKAYHEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQFGPVALNPA TLLPLPEEGAPHDCEILAEHTGTEPDLTDQPIPADHTWYTDGSSFLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYKRRGGLTSEGKEIKNKSEILALLKALFLPKRLSIHCLGHQKGDSAEARGNRLADQAAREAAIKTPPDTSTLL
MLVRD _P1122 7_3mut	TLNIEDEYRLHEISTEPDVSPGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIIKQYPMSEARLGKIPHIQRLDQGLVPCQSPWNTPL LPVKKPGTNDYRVPVQDLREVNRVEDIHPTVNPYNLLSGLPTSHRWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPGEMGISGQLTWTRLPQGFK NSPTLFNEALHRGLADFRIQHPDLILLQYVDDLLAATSELDCQQGTRALLQTLGNLGYRASAKKQICQKQVKYLYLLREGQRWLTEARKETVMG QPTPKTPRQLREFLGKAGFCRLWIPRFAEMAAPLYPLTKPGTLFNWGPDDQKAYHEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQLG PWRRPVAYLSKKLDPVAGWPPCLRMVAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQFGPVALNPA TLLPLPEEGAPHDCEILAEHTGTEPDLTDQPIPADHTWYTDGSSFLQEGQRKAGAAVTETETEVIAKALPAGTSAQRAELIALTQALKMAEGKKL NVYTDSTRYAFATAHIHGEIYKRRGGLTSEGKEIKNKSEILALLKALFLPKRLSIHCLGHQKGDSAEARGNRLADQAAREAAIKTPPDTSTLL
MMTVB _P0336 5	WVQEISDSRPMILHIYNGRRFLGLLNTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDI MKDIKVRMLMTDSPDDSQDLMIGAESNLFADQISWKSQDPVWLNQWPLKQEKALQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQD LRAVNATMHDMGALQPGLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSPTLCQKFVDKAILTVRD KYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTIQQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKT TGELKPLFEILNGDSNPISSTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTACLWQDGVVEWHLPHISPKVITPYDIFCTQLIKG RHRSELFSKDPDYPYVYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLFTLQTAIIFPHMTSTTLEKGVIFTDGSANGRSVYIQGREPI KENTQNTAQQAIEVAVITAFEEVSQPFNLTYDSKYVTGLFPEIETATLSPRTKIYTELKHLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRI LT
MMTVB _P0336 5	WVQEISDSRPMILHIYNGRRFLGLLNTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDI MKDIKVRMLMTDSPDDSQDLMIGAESNLFADQISWKSQDPVWLNQWPLKQEKALQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQD LRAVNATMHDMGALQPGLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSPTLCQKFVDKAILTVRD KYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTIQQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKT TGELKPLFEILNGDSNPISSTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTACLWQDGVVEWHLPHISPKVITPYDIFCTQLIKG RHRSELFSKDPDYPYVYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLFTLQTAIIFPHMTSTTLEKGVIFTDGSANGRSVYIQGREPI KENTQNTAQQAIEVAVITAFEEVSQPFNLTYDSKYVTGLFPEIETATLSPRTKIYTELKHLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRI LT
MMTVB _P0336 5_2mut	WVQEISDSRPMILHIYNGRRFLGLLNTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDI MKDIKVRMLMTDSPDDSQDLMIGAESNLFADQISWKSQDPVWLNQWPLKQEKALQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQD LRAVNATMHDMGALQPGLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSPTLCQKFVDKAILTVRD KYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTIQQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKT TGELKPLFEILNPDSPISSTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTACLWQDGVVEWHLPHISPKVITPYDIFCTQLIKG RHRSELFSKDPDYPYVYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLFTLQTAIIFPHMTSTTLEKGVIFTDGSANGRSVYIQGREPI KENTQNTAQQAIEVAVITAFEEVSQPFNLTYDSKYVTGLFPEIETATLSPRTKIYTELKHLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRI LT

RT Name	RT amino acid sequence
MMTVB_P0336_5_2mut_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5_2mut_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5_2mut_B	WVQEISDSRPMILHIYLNRRFLGLLNTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM MKDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD LRAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK KYQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT TGELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK KENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRI LT
MMTVB_P0336_5_2mut_B	WVQEISDSRPMILHIYLNRRFLGLLNTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM MKDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD LRAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK KYQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT TGELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK KENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRI LT
MMTVB_P0336_5_2mut_B_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5_2mut_B_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5_WS	VQEISDSRPMILHIYLNRRFLGLLDTGADKTCIAGRDWPANWPIHQTESSLQGLGMACGVARSSQPLRWQHEDKSGIIHPFVIPTLPFTLWGRDIM KDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRLQLD RAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAILTVRDK YQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFLKLT GELKPLFEILNPDSPNISTRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYTPTAQLWQDGVVEWIHLPHISPKVITPYDIFCTQLIIKGR HRSKELFSKDPDYIVVPYTKVQFDLLLQEKEDWPISLLGFLGEVHFHLPKDPLLTFTLQTALIFPHMTSTTPLEKGIVIFTDGSANGRSVITYIQGREPIIK ENTQNTAQQAIEVAVITAFEEVSQPFNLYTDSKYVTGLFPEIETATLSPRTKIYTELKHQLRIHKKRQEKFYIGHIRGHTGLPGPLAQGNAYADSLTRIL TA
MMTVB_P0336_5-Pro	GRDIMKDIKVRMLMTSDPDDSQDLMIGAIESNLFADQISWKSQDPVWLNQWPLKQEKQLALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWRL LLQDLRAVNATMHDMGALQPLPSPVAVPKGWEIIIDLQDCFFNIKHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPFL

RT Name	RT amino acid sequence
	LKLTTGELKPLFEILNGDSNPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MMTVB _P0336 5-Pro	GRDIMKDIKVRLMTDSPDSDQLMIGAIENLFDQISWKSQDPVWLNQWPLKQEKQLQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWR LLQDLRAVNATMHDGALQPLSPVAVPKGWEIILDLQDCCFNKILHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPF LKLTTGELKPLFEILNGDSNPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MMTVB _P0336 5- Pro_2m ut	GRDIMKDIKVRLMTDSPDSDQLMIGAIENLFDQISWKSQDPVWLNQWPLKQEKQLQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWR LLQDLRAVNATMHDGALQPLSPVAVPKGWEIILDLQDCCFNKILHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPF LKLTTGELKPLFEILNPDSPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MMTVB _P0336 5- Pro_2m ut	GRDIMKDIKVRLMTDSPDSDQLMIGAIENLFDQISWKSQDPVWLNQWPLKQEKQLQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWR LLQDLRAVNATMHDGALQPLSPVAVPKGWEIILDLQDCCFNKILHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPF LKLTTGELKPLFEILNPDSPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MMTVB _P0336 5- Pro_2m utB	GRDIMKDIKVRLMTDSPDSDQLMIGAIENLFDQISWKSQDPVWLNQWPLKQEKQLQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWR LLQDLRAVNATMHDGALQPLSPVAPPKGWEIILDLQDCCFNKILHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPF LKLTTGELKPLFEILNPDSPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MMTVB _P0336 5- Pro_2m utB	GRDIMKDIKVRLMTDSPDSDQLMIGAIENLFDQISWKSQDPVWLNQWPLKQEKQLQALQQLVTEQLQLGHLEESNSPWNTPVFVIKKKSGKWR LLQDLRAVNATMHDGALQPLSPVAPPKGWEIILDLQDCCFNKILHPEDCKRFAFSVSPNFKRPYQRFQWKVLPQGMKNSTPLCQKFVDKAIL TVRDKYQDSYIVHYMDILLAHPSRSIVDEILTSMIQALNKHGLVSTEKIQKYDNLKYLGTTHIQGDSVSYQKLQIRTDKRLTNDFQKLLGNINWIRPF LKLTTGELKPLFEILNPDSPISIRKLTPEACKALQLMNERLSTARVKRLDLSQPWSLCILKTEYPTACLWQDGVVEWIHLPHISPKVITPYDICTQL IIKGRHRSKELFSKDPDYIVVPYTKVQFDLLQEKEDWPISLLGFLGEVHFHLPKDPDLLTFTLQTAIIFPHMTSTTPLEKGIVIFDGSANGRSVTYIQGR EPIIKENTQNTAQQAIEAVITAFEEVSQPFNLVYDTSKYVTGLFPEIETATLSPRTKIYTELKHLLQRLIHKRQEKFYIGHIRGHTGLPGPLAQGNAYADSL TRILT
MPMV _P07572	LTAADILAPQCCAEPIWKSDEPVWVDQWPLTNDKLAQAQQLVQEQLEAGHITESSPWNTPIFVIKKKSGKWRLLQDLRAVNATMVMGALQPG LPSPVAPPQGYLKIIDLKDCFFSIPLHPSDQKRFAPSLSTNFKPMQRFQWKVLPQGMANSPTLCQKYVATAIHKVRHAWKQMYIHYMDILLIAG DGGQVLQCFDQLKQELTAAGLHIAPEKVQLQDPYTYLGFELNGPKITNQKAVIRKDKLQTLNDFQKLLGDNWLRPYLKLTTGDLKPLFDLTKGDS PNSHRSLKEALASLEKVEIAEQFVTHINYSPLIFLIFNTALTPTGLFWQDNPMWIHLPASPKKVLPPYYDAIADLILGRDHSSKYFGEIPSTIOP YSKSQIDWLMQNTMEMWPIACASFVGLDNHYPPNKLIFQCKLHTFVFPQIISKTLNNALLVFTDGSSTGMAAYTLTDTTIKFQTLNLSAQLVELQALIA VLSAFPNNQLNIYDTSAYLAHSIPLLETVAQIKHISSETAKLFLCQQLIYNRSIPFYIGHVRAHSGLPPIAQGNQADLTKIVASNNIT
MPMV _P07572 _2mutB	LTAADILAPQCCAEPIWKSDEPVWVDQWPLTNDKLAQAQQLVQEQLEAGHITESSPWNTPIFVIKKKSGKWRLLQDLRAVNATMVMGALQPG LPSPVAPPQGYLKIIDLKDCFFSIPLHPSDQKRFAPSLSTNFKPMQRFQWKVLPQGMANSPTLCQKYVATAIHKVRHAWKQMYIHYMDILLIAG KDGQQLVQCFDQLKQELTAAGLHIAPEKVQLQDPYTYLGFELNGPKITNQKAVIRKDKLQTLNDFQKLLGDNWLRPYLKLTTGDLKPLFDLTKPDS DPNSHRSLKEALASLEKVEIAEQFVTHINYSPLIFLIFNTALTPTGLFWQDNPMWIHLPASPKKVLPPYYDAIADLILGRDHSSKYFGEIPSTIOP YSKSQIDWLMQNTMEMWPIACASFVGLDNHYPPNKLIFQCKLHTFVFPQIISKTLNNALLVFTDGSSTGMAAYTLTDTTIKFQTLNLSAQLVELQALIA VLSAFPNNQLNIYDTSAYLAHSIPLLETVAQIKHISSETAKLFLCQQLIYNRSIPFYIGHVRAHSGLPPIAQGNQADLTKIVASNNIT
PERV _Q4VFZ 2	TLQLDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLIQQGILVPVQSPWNT LLPVKPGTNDYRPFVQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPQGF KNSPTIFDEALHRDLANFRIQHPQVTLQYVDDLLLAGATKQDCLEGTAKLLELSDLYRASAKKAQICREVITYLGYSLRDGQRWLTEARKKTIV QIPAPTTAKQVREFLGTAGFRLWIPGFATLAAPLYLTKEKGEFSWAPEHQKAFDAIKKALLSAPALAPDVTKPFTLYVDERKGVARGVLTQTLGP WRRPVAYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGLQNITVIAPHALENIVRQPPDRWMTNARMTHYSLLTERTVTFAPPAALNPATLLPE ETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYVECKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALQALRLAEGKSINIYT DSRYAFATAHVHGAIKYQGRLLTSAGREIKNKEEILSLEALHLPKRLAIHCPCGHQKAKDPISRGNMADRVAQQAQGVNLL
PERV _Q4VFZ 2	TLQLDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLIQQGILVPVQSPWNT LLPVKPGTNDYRPFVQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPQGF KNSPTIFDEALHRDLANFRIQHPQVTLQYVDDLLLAGATKQDCLEGTAKLLELSDLYRASAKKAQICREVITYLGYSLRDGQRWLTEARKKTIV QIPAPTTAKQVREFLGTAGFRLWIPGFATLAAPLYLTKEKGEFSWAPEHQKAFDAIKKALLSAPALAPDVTKPFTLYVDERKGVARGVLTQTLGP WRRPVAYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGLQNITVIAPHALENIVRQPPDRWMTNARMTHYSLLTERTVTFAPPAALNPATLLPE ETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYVECKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALQALRLAEGKSINIYT DSRYAFATAHVHGAIKYQGRLLTSAGREIKNKEEILSLEALHLPKRLAIHCPCGHQKAKDPISRGNMADRVAQQAQGVNLL

RT Name	RT amino acid sequence
PERV_Q4VFZ_2_3mut	TLQLDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLLIQGGILVPVQSPWNTPLPVKPGTNDYRVPQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPGQFKNSPTIFNEALHRDLANFRIQHPQVTLQYVDDLLAGATKQDCEGTAKLLLESDLGYRASAKKAQICRREVITYLGYSLRQDQGRWLTEARKKTVVQIPAPTTAKQVREFLGTAGFCRLWIPGFATLAAPLYPLTKPKGEFSWAPEHQKAFDAIKKALLSAPALALPDVTKPFITYLDERKCVARGVLTQTLGPRVAVYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGQNTIVIAPHALENIVRQPPDRWMTNARMTHYQSLLLTERVTFAPPAALNPATLLPEETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYYVEGKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALTQALRLAEGKSINIYTDSDRYAFATAHVGAIYKQRGWLTSGAGREIKNKEEILSLEALHLPKRLAIHCPGHQKAKDPISRGNQMDRVAQKAAQGVNLL
PERV_Q4VFZ_2_3mut	TLQLDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLLIQGGILVPVQSPWNTPLPVKPGTNDYRVPQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPGQFKNSPTIFNEALHRDLANFRIQHPQVTLQYVDDLLAGATKQDCEGTAKLLLESDLGYRASAKKAQICRREVITYLGYSLRQDQGRWLTEARKKTVVQIPAPTTAKQVREFLGTAGFCRLWIPGFATLAAPLYPLTKPKGEFSWAPEHQKAFDAIKKALLSAPALALPDVTKPFITYLDERKCVARGVLTQTLGPRVAVYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGQNTIVIAPHALENIVRQPPDRWMTNARMTHYQSLLLTERVTFAPPAALNPATLLPEETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYYVEGKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALTQALRLAEGKSINIYTDSDRYAFATAHVGAIYKQRGWLTSGAGREIKNKEEILSLEALHLPKRLAIHCPGHQKAKDPISRGNQMDRVAQKAAQGVNLL
PERV_Q4VFZ_2_3mut_A_WS	LDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLLIQGGILVPVQSPWNTPLLPVRKPGTNDYRVPQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPGQFKNSPTIFNEALHRDLANFRIQHPQVTLQYVDDLLAGATKQDCEGTAKLLLESDLGYRASAKKAQICRREVITYLGYSLRQDQGRWLTEARKKTVVQIPAPTTAKQVREFLGTAGFCRLWIPGFATLAAPLYPLTKPKGEFSWAPEHQKAFDAIKKALLSAPALALPDVTKPFITYLDERKCVARGVLTQTLGPRVAVYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGQNTIVIAPHALENIVRQPPDRWMTNARMTHYQSLLLTERVTFAPPAALNPATLLPEETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYYVEGKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALTQALRLAEGKSINIYTDSDRYAFATAHVGAIYKQRGWLTSGAGREIKNKEEILSLEALHLPKRLAIHCPGHQKAKDPISRGNQMDRVAQKAAQGVNLL
PERV_Q4VFZ_2_3mut_A_WS	LDDEYRLYSPLVKPDQNIQFWLEQFPQAWAETAGMGLAKQVPPQVQLKASATPVSVRQYPLSKEAQEGIRPHVQRLLIQGGILVPVQSPWNTPLLPVRKPGTNDYRVPQDLREVNRVQDIHPTVPNPYNLLCALPPQRSWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPGTGRTGQLTWTRLPGQFKNSPTIFNEALHRDLANFRIQHPQVTLQYVDDLLAGATKQDCEGTAKLLLESDLGYRASAKKAQICRREVITYLGYSLRQDQGRWLTEARKKTVVQIPAPTTAKQVREFLGTAGFCRLWIPGFATLAAPLYPLTKPKGEFSWAPEHQKAFDAIKKALLSAPALALPDVTKPFITYLDERKCVARGVLTQTLGPRVAVYLSKKLDPVAGSWPVCLKAIAAVAILVKDADKLTGQNTIVIAPHALENIVRQPPDRWMTNARMTHYQSLLLTERVTFAPPAALNPATLLPEETDEPVTHDCHQLLIEETGVRKDLTDIPLTGEVLTWFTDGSYYVEGKRMAGAAVVDGTRTIWASSLPEGTSQAQKAEALMALTQALRLAEGKSINIYTDSDRYAFATAHVGAIYKQRGWLTSGAGREIKNKEEILSLEALHLPKRLAIHCPGHQKAKDPISRGNQMDRVAQKAAQGVNLL
SFV1_P23074	MDPLQLLQPLEAEIKGTGLKAHWNAGATITCVPEAFLEDERPQITMLTIHGEKQQDVYYLTFKVQGRKVEAEVLASPYDYILLNPDSVPWLMMKKPLQLTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIKPHNIATGLAPRPQKQYPINPKAKPSIQIVIDLLKQGVLIQONSMTMTVPVVPKPDGKWRMVL DYREVNKTIPIAAQNQHSAGILSSYRGKYKTTLDLTNGFWAHPITPESYWLTAFTWQKQYCWTRLPGGFLNSPALFTADVVDLLKEIPNVQAYVDDIYSHDDPQEHLEQLEKIFISILLNAGYVVSLLKKEIAQREVEFLGFNITKEGRGLTDTFKQKLLNITPPKDLKQLQSI LGLNLFARNFIPNYSSELVKPLYTIVANANGKFIWTEEDNSNQLQHISVLNQADNLEERNPETRLIIVNSSPSAGYIRYNEGSKRPMYVNYIFSKAE AKFTQTEKLLTMMHGLIKAMDLMGQELVYSPVSMTKIQRTPPERKALPVRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDEVIKHPSEFAMVYFDGSAIKHPDVNKSAGMGIAQVQFIPEYKIVHQWSIPLGDHTAQLAEIAAEVAFACKKALKISGPVLIVTDSFYVAESANKELPYWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMHEKGHQQPMITLHTEGNNLADKLATQGSYYVH
SFV1_P23074_2mut	MDPLQLLQPLEAEIKGTGLKAHWNAGATITCVPEAFLEDERPQITMLTIHGEKQQDVYYLTFKVQGRKVEAEVLASPYDYILLNPDSVPWLMMKKPLQLTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIKPHNIATGLAPRPQKQYPINPKAKPSIQIVIDLLKQGVLIQONSMTMTVPVVPKPDGKWRMVL DYREVNKTIPIAAQNQHSAGILSSYRGKYKTTLDLTNGFWAHPITPESYWLTAFTWQKQYCWTRLPGGFLNSPALFTADVVDLLKEIPNVQAYVDDIYSHDDPQEHLEQLEKIFISILLNAGYVVSLLKKEIAQREVEFLGFNITKEGRGLTDTFKQKLLNITPPKDLKQLQSI LGLNLFARNFIPNYSSELVKPLYTIVANANGKFIWTEEDNSNQLQHISVLNQADNLEERNPETRLIIVNSSPSAGYIRYNEGSKRPMYVNYIFSKAE AKFTQTEKLLTMMHGLIKAMDLMGQELVYSPVSMTKIQRTPPERKALPVRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDEVIKHPSEFAMVYFDGSAIKHPDVNKSAGMGIAQVQFIPEYKIVHQWSIPLGDHTAQLAEIAAEVAFACKKALKISGPVLIVTDSFYVAESANKELPYWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMHEKGHQQPMITLHTEGNNLADKLATQGSYYVH
SFV1_P23074_2mutA	MDPLQLLQPLEAEIKGTGLKAHWNAGATITCVPEAFLEDERPQITMLTIHGEKQQDVYYLTFKVQGRKVEAEVLASPYDYILLNPDSVPWLMMKKPLQLTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIKPHNIATGLAPRPQKQYPINPKAKPSIQIVIDLLKQGVLIQONSMTMTVPVVPKPDGKWRMVL DYREVNKTIPIAAQNQHSAGILSSYRGKYKTTLDLTNGFWAHPITPESYWLTAFTWQKQYCWTRLPGGFLNSPALFTADVVDLLKEIPNVQAYVDDIYSHDDPQEHLEQLEKIFISILLNAGYVVSLLKKEIAQREVEFLGFNITKEGRGLTDTFKQKLLNITPPKDLKQLQSI LGLNLFARNFIPNYSSELVKPLYTIVANANGKFIWTEEDNSNQLQHISVLNQADNLEERNPETRLIIVNSSPSAGYIRYNEGSKRPMYVNYIFSKAE AKFTQTEKLLTMMHGLIKAMDLMGQELVYSPVSMTKIQRTPPERKALPVRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDEVIKHPSEFAMVYFDGSAIKHPDVNKSAGMGIAQVQFIPEYKIVHQWSIPLGDHTAQLAEIAAEVAFACKKALKISGPVLIVTDSFYVAESANKELPYWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMHEKGHQQPMITLHTEGNNLADKLATQGSYYVH
SFV1_P23074-Pro	VPWLMMKKPLQLTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIKPHNIATGLAPRPQKQYPINPKAKPSIQIVIDLLKQGVLIQONSMTMTVPVVPKPDGKWRMVL DYREVNKTIPIAAQNQHSAGILSSYRGKYKTTLDLTNGFWAHPITPESYWLTAFTWQKQYCWTRLPQGFLNSPALFTADVVDLLKEIPNVQAYVDDIYSHDDPQEHLEQLEKIFISILLNAGYVVSLLKKEIAQREVEFLGFNITKEGRGLTDTFKQKLLNITPPKDLKQLQSI LGLNLFARNFIPNYSSELVKPLYTIVANANGKFIWTEEDNSNQLQHISVLNQADNLEERNPETRLIIVNSSPSAGYIRYNEGSKRPMYVNYIFSKAEAKFTQTEKLLTMMHGLIKAMDLMGQELVYSPVSMTKIQRTPPERKALPVRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDEVIKHPSEFAMVYFDGSAIKHPDVNKSAGMGIAQVQFIPEYKIVHQWSIPLGDHTAQLAEIAAEVAFACKKALKISGPVLIVTDSFYVAESANKELPYWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMHEKGHQQPMITLHTEGNNLADKLATQGSYYVH
SFV1_P23074-Pro_2mut	VPWLMMKKPLQLTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIKPHNIATGLAPRPQKQYPINPKAKPSIQIVIDLLKQGVLIQONSMTMTVPVVPKPDGKWRMVL DYREVNKTIPIAAQNQHSAGILSSYRGKYKTTLDLTNGFWAHPITPESYWLTAFTWQKQYCWTRLPQGFLNSPALFTADVVDLLKEIPNVQAYVDDIYSHDDPQEHLEQLEKIFISILLNAGYVVSLLKKEIAQREVEFLGFNITKEGRGLTDTFKQKLLNITPPKDLKQLQSI LGLNLFARNFIPNYSSELVKPLYTIVANANGKFIWTEEDNSNQLQHISVLNQADNLEERNPETRLIIVNSSPSAGYIRYNEGSKRPMYVNYIFSKAEAKFTQTEKLLTMMHGLIKAMDLMGQELVYSPVSMTKIQRTPPERKALPVRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDEVIKHPSEFAMVYFDGSAIKHPDVNKSAGMGIAQVQFIPEYKIVHQWSIPLGDHTAQLAEIAAEVAFACKKALKISGPVLIVTDSFYVAESANKELPYWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMHEKGHQQPMITLHTEGNNLADKLATQGSYYVH

RT Name	RT amino acid sequence
SFV1_ P23074 - Pro_2mutA	VPWLMKKPLQLTTLVPLHEYQERLLQQTALPKEQKELLQKFLKYDALWQHWENQVGHRRIRKPHNIATGTLAPRPQKQYPINPKAKPSIQVINDLLK QGVLIQQNSTMNTVPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWQQGQYCWTR LPQGFLNSPALFNADVVDLLKEIPNVQVYVDDIYSHDDPREHLEQLEKIFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTDTFKQKLLNITPP KDLKQLQSILGKLNFAFNIPNFSELVKPLYNIATAPANGKIFSWTEDNSNQLQHIISVLNQADNLEERNPETRIKLVNSSPSAGYIRYVNEGSKRPIMY VNYIFSKAFAKFTQTEKLLTTHMKGLIKAMDLAGMQEILVYSPVSMTKIQRTPLPERKALPIRWITWMTYLEDPRIQFHYDKSLPELQQIPNVTEDVI AKTKHPSEFAMFVYTDGSAIKHPDNVNSHAGMAGIAQVQFPEYKIVHQSISPLGDHTAQLAEIAAEVFAACKKALKISGPNVITDSFYVAESANKELP YWKSNGFLNNKKPLRHVSKWKSIAECLQLKPDIIIMEKHGQHPMTTLHTEGNNLADKLATQGSYYVN
SFV3L_ P27401	MDPLQLLQPLAEIAGTKLKAHWN SGATITCVPQAFLEEEVPIKNWKTTHGEKEQPYYLTFKIQGRKVEAEVISSPYDYLVS PDIPLWLMKKPLQL TTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLKQGVLIQQNSIM NTPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTRLPQGFLNSPAL FTADVVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNITPPRDLKQLQSIL GLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPIMLYNYVYTKAEV KFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDIIAKIKHPSEFSMV FYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKELPYWQSGNGFFNN KKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFV3L_ P27401 _2mut	MDPLQLLQPLAEIAGTKLKAHWN SGATITCVPQAFLEEEVPIKNWKTTHGEKEQPYYLTFKIQGRKVEAEVISSPYDYLVS PDIPLWLMKKPLQL TTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLKQGVLIQQNSIM NTPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTRLPQGFLNSPAL FNADVVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNITPPRDLKQLQSIL GLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPIMLYNYVYTKAEV KFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDIIAKIKHPSEFSMV FYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKELPYWQSGNGFFNN KKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFV3L_ P27401 _2mutA	MDPLQLLQPLAEIAGTKLKAHWN SGATITCVPQAFLEEEVPIKNWKTTHGEKEQPYYLTFKIQGRKVEAEVISSPYDYLVS PDIPLWLMKKPLQL TTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLKQGVLIQQNSIM NTPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTRLPQGFLNSPAL FNADVVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNITPPRDLKQLQSIL GLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPIMLYNYVYTKAEV KFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDIIAKIKHPSEFSMV FYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKELPYWQSGNGFFNN KKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFV3L_ P27401 -Pro	IPWLMKKPLQLTTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLK QGVLIQQNSIMNTVPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTR LPQGFLNSPALFADVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNIT PPRDLKQLQSILGLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPI MYLNYVYTKAEVKFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDII AKIKHPSEFSMVFYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKEL PYWQSGNGFFNNKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFV3L_ P27401 - Pro_2mut	IPWLMKKPLQLTTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLK QGVLIQQNSIMNTVPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTR LPQGFLNSPALFNADVVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNIT PPRDLKQLQSILGLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPI MYLNYVYTKAEVKFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDII AKIKHPSEFSMVFYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKEL PYWQSGNGFFNNKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFV3L_ P27401 - Pro_2mutA	IPWLMKKPLQLTTLVPLQEYERLLKQTMLTGSYKEKLQSLFLKYDALWQHWENQVGHRRIRKPHIATGTVNPRPQKQYPINPKAKASIQTVINDLLK QGVLIQQNSIMNTVPVVPKPDGKWRMVL DYREVNTIPLIAAQNHQSAGILSSIFRGKYKTTLDLNSGFWAHSITPESYWLTAFTWLGQQYCWTR LPQGFLNSPALFNADVVDLLKEVPNVQVYVDDIYSHDDPREHLEQLEKVFSLNAGYVVSLLKKSEIAQHEVEFLGFNITKEGRGLTETFKQKLLNIT PPRDLKQLQSILGLLNFARNFIPNFSELVKPLYNIATAPGKYITWTTDNSQQLQNIISMLNSAENLEERNPEVRLIMKVNTSPSAGYIRFYNEFAKRPI MYLNYVYTKAEVKFTNTEKLLTTHMKGLIKALDLMGQEILVYSPVSMTKIQKTPLPERKALPIRWITWMSYLEDPRIQFHYDKTLPQLQQVPTVTDII AKIKHPSEFSMVFYTDGSAIKHPNVNKS HNAGMAGIAQVQFPEFTVINTWSIPLGDHTAQLAEVAAVEFAACKKALKIDGPVLIVTDSFYVAESVNKEL PYWQSGNGFFNNKKPLKHVSKWKSIADCIQLKPDIIIMEKHGQHTASTFHTTEGNNLADKLATQGSYYVN
SFVCP _Q8704 0	MNPLQLLQPLPAEVKGTLLAHWN SGATITCIPESFLEDEQPIKQTLIKTHGEKQNNVYLLTFKVKGRKVEAEVIASPYEYILLSPDTPWLTQQPLQ LTLVPLQEYQDRILNKALPEEQKQLKALFTKYDNLWQHWENQVGHRRIRKPHIATGDYPPRPQKQYPINPKAKPSIQVINDLLKQGVLTQPNST MNTVPVVPKPDGGRWRMVL DYREVNTIPLTAAQNHQSAGILATVIRQKYKTTDLANGFWAHSITPDSYWLTAFTWQQGQYCWTRLPQGFLNSP ALFTADVVDLLKEVPNVQVYVDDIYSHDNPFHEIQLEKVFQILLQAGYVVSLLKKSEIGQRTVEFLGFNITKEGRGLTDTFKTKLLNVTTPPKDLKQLQ SILGLLNFARNFIPNFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSAGYVRYNYESGKKPIMLYNVVFSK AELKFSMLEKLLTTHMKALIKAMDLAGMQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPQLKHIPDVYTSIPPLKHPSQ YEGVCTDGSIAKSPDPTKSNAGMIGVHAIYNEPYKILNQWSIPLGHHTAQMAEIAAEVFAACKKALKVGPVLIVTDSFYVAESANKELPYWKSNGF VNNKKEPLKHISKWSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYYVN
SFVCP _Q8704 0_2mut	MNPLQLLQPLPAEVKGTLLAHWN SGATITCIPESFLEDEQPIKQTLIKTHGEKQNNVYLLTFKVKGRKVEAEVIASPYEYILLSPDTPWLTQQPLQ LTLVPLQEYQDRILNKALPEEQKQLKALFTKYDNLWQHWENQVGHRRIRKPHIATGDYPPRPQKQYPINPKAKPSIQVINDLLKQGVLTQPNST MNTVPVVPKPDGGRWRMVL DYREVNTIPLTAAQNHQSAGILATVIRQKYKTTDLANGFWAHSITPDSYWLTAFTWQQGQYCWTRLPQGFLNSP ALFNADVVDLLKEVPNVQVYVDDIYSHDNPFHEIQLEKVFQILLQAGYVVSLLKKSEIGQRTVEFLGFNITKEGRGLTDTFKTKLLNVTTPPKDLKQLQ SILGLLNFARNFIPNFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSAGYVRYNYESGKKPIMLYNVVFSK AELKFSMLEKLLTTHMKALIKAMDLAGMQEILVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPQLKHIPDVYTSIPPLKHPSQ

RT Name	RT amino acid sequence
	YEGVCTDGSIAKSPDPTKSNAGMGIVHAIYNPEYKILNQWSIPLGHHTAQMAEIAAEVAFACKKALKVPGPVLVITDSFYVAESANKELPYWKSNGFVNNKKEPLKHISKWKSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYVNN
SFVCP_Q8704_0_2mut A	MNPLQLQLPAEVKGTLLAHWNISGATITCIPESFLEDEQPIKQTLIKTHGEKQNNVYYLTFKVKGRKVEAEVIASPYEYILLSPDTPVWLTQQPLQLTILVPLQEYQDRILNKTALPEEQKQQLKALFTKYDNLWQHWNQVGHRIKIRPHNIATGDYPPRPQKQYPINPKAKPSIQIIDDLLKQGVLTQPNSTMNTPVVPKPDGRWRMVL DYREVNKTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPDSYWLTAFTWQKGQYCWTRLPQGFLNSPALFNADAVDLLKEVPNVQVYVDDIYLSHDNPHEHIQLEKVFQILLQAGYVVSLLKKEIGQRTVEFLGNITKEGRGLTDTFTKLLNVTTPPKDLKQLQ SILGKLNFAFNFIPIFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSPSAGYVRYNYESGKKPIMYLYNVFSKAEKFSMLEKLLTTMHKALIKAMDAMGQELVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELHIPDVYTSIPPLKHPSPQYEGVCTDGSIAKSPDPTKSNAGMGIVHAIYNPEYKILNQWSIPLGHHTAQMAEIAAEVAFACKKALKVPGPVLVITDSFYVAESANKELPYWKSNGFVNNKKEPLKHISKWKSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYVNN
SFVCP_Q8704_0-Pro	VPWLTPQQPLQLTILVPLQEYQDRILNKTALPEEQKQQLKALFTKYDNLWQHWNQVGHRIKIRPHNIATGDYPPRPQKQYPINPKAKPSIQIIDDLLKQGVLTQPNSTMNTPVVPKPDGRWRMVL DYREVNKTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPDSYWLTAFTWQKGQYCWTRLPQGFLNSPALFNADAVDLLKEVPNVQVYVDDIYLSHDNPHEHIQLEKVFQILLQAGYVVSLLKKEIGQRTVEFLGNITKEGRGLTDTFTKLLNVTTPPKDLKQLQ SILGKLNFAFNFIPIFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSPSAGYVRYNYESGKKPIMYLYNVFSKAEKFSMLEKLLTTMHKALIKAMDAMGQELVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELHIPDVYTSIPPLKHPSPQYEGVCTDGSIAKSPDPTKSNAGMGIVHAIYNPEYKILNQWSIPLGHHTAQMAEIAAEVAFACKKALKVPGPVLVITDSFYVAESANKELPYWKSNGFVNNKKEPLKHISKWKSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYVNN
SFVCP_Q8704_0-Pro_2mut	VPWLTPQQPLQLTILVPLQEYQDRILNKTALPEEQKQQLKALFTKYDNLWQHWNQVGHRIKIRPHNIATGDYPPRPQKQYPINPKAKPSIQIIDDLLKQGVLTQPNSTMNTPVVPKPDGRWRMVL DYREVNKTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPDSYWLTAFTWQKGQYCWTRLPQGFLNSPALFNADAVDLLKEVPNVQVYVDDIYLSHDNPHEHIQLEKVFQILLQAGYVVSLLKKEIGQRTVEFLGNITKEGRGLTDTFTKLLNVTTPPKDLKQLQ SILGKLNFAFNFIPIFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSPSAGYVRYNYESGKKPIMYLYNVFSKAEKFSMLEKLLTTMHKALIKAMDAMGQELVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELHIPDVYTSIPPLKHPSPQYEGVCTDGSIAKSPDPTKSNAGMGIVHAIYNPEYKILNQWSIPLGHHTAQMAEIAAEVAFACKKALKVPGPVLVITDSFYVAESANKELPYWKSNGFVNNKKEPLKHISKWKSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYVNN
SFVCP_Q8704_0-Pro_2mutA	VPWLTPQQPLQLTILVPLQEYQDRILNKTALPEEQKQQLKALFTKYDNLWQHWNQVGHRIKIRPHNIATGDYPPRPQKQYPINPKAKPSIQIIDDLLKQGVLTQPNSTMNTPVVPKPDGRWRMVL DYREVNKTIPLTAAQNQHSAGILATVRQKYKTTLDLANGFWAHPITPDSYWLTAFTWQKGQYCWTRLPQGFLNSPALFNADAVDLLKEVPNVQVYVDDIYLSHDNPHEHIQLEKVFQILLQAGYVVSLLKKEIGQRTVEFLGNITKEGRGLTDTFTKLLNVTTPPKDLKQLQ SILGKLNFAFNFIPIFAELVQTLNYLIASSPGKYIEWTEDNTKQLNKVIEALNTASNLEERLPDQRLVIKVNTPSPSAGYVRYNYESGKKPIMYLYNVFSKAEKFSMLEKLLTTMHKALIKAMDAMGQELVYSPVSMTKIQKTPLPERKALPIRWITWMTYLEDPRIQFHYDKTLPKELHIPDVYTSIPPLKHPSPQYEGVCTDGSIAKSPDPTKSNAGMGIVHAIYNPEYKILNQWSIPLGHHTAQMAEIAAEVAFACKKALKVPGPVLVITDSFYVAESANKELPYWKSNGFVNNKKEPLKHISKWKSIAECLSIKPDITIQHEKGHPINTSIHTEGNALADKLATQGSYVNN
SMRVH_P0336_4	PRSRADIPVPHADKISWKITDPVWVDQWPLTYEKTAAIALVQEQLAAGHIEPTNSPWNTPIFIKKKSGSWRLLQDLRAVNKVMVPMGALQPGPLSPVAIPLNYHKIVIDLKDCFFTIPLHPEDRPYFAFSVPQINFQSPMPRYQWKVLPQGMANSPTLCQKFVAAAIAPVRSQWPEAYILHYMDILLACDSA EAAKACYAHIISCLTSYGLKIAPDKVQVSEPFYSLGFELHHQVFTPRVCLKTDHLKTLNDFQKLLGDIQWLRPYLKLPTSAVLPLNLIKGDPNPLSV RALTPKAEKQSLALINKAIQNQSVQGISYNLPLVLLLTPTHTPTAVFWQPNGTDPKNGSPLLWLHLPASPSKVLLTYPSSLAMLIIKGRYTRGQLFGRDPHSIIIFYTQDQLTWLLQTSDEWAIALSSFTGDIDNHYSPDPVIAQFALKHQFIFPKITKCAPIQATLVFTDGSNGIAAYVIDNQPSIKSPYLSAQLVE LYAILQVFTVLAHQPFNLTYDSAYIAQSVPLLETVPFIKSSSTNATPLFSKLQQLILNRQHPFFIGHLRAHLNLPGLPACGNALADAATQIFPIISD
SMRVH_P0336_4_2mut	PRSRADIPVPHADKISWKITDPVWVDQWPLTYEKTAAIALVQEQLAAGHIEPTNSPWNTPIFIKKKSGSWRLLQDLRAVNKVMVPMGALQPGPLSPVAIPLNYHKIVIDLKDCFFTIPLHPEDRPYFAFSVPQINFQSPMPRYQWKVLPQGMANSPTLCQKFVAAAIAPVRSQWPEAYILHYMDILLACDSA EAAKACYAHIISCLTSYGLKIAPDKVQVSEPFYSLGFELHHQVFTPRVCLKTDHLKTLNDFQKLLGDIQWLRPYLKLPTSAVLPLNLIKGDPNPLSV RALTPKAEKQSLALINKAIQNQSVQGISYNLPLVLLLTPTHTPTAVFWQPNGTDPKNGSPLLWLHLPASPSKVLLTYPSSLAMLIIKGRYTRGQLFGRDPHSIIIFYTQDQLTWLLQTSDEWAIALSSFTGDIDNHYSPDPVIAQFALKHQFIFPKITKCAPIQATLVFTDGSNGIAAYVIDNQPSIKSPYLSAQLVE LYAILQVFTVLAHQPFNLTYDSAYIAQSVPLLETVPFIKSSSTNATPLFSKLQQLILNRQHPFFIGHLRAHLNLPGLPACGNALADAATQIFPIISD
SMRVH_P0336_4_2mut B	PRSRADIPVPHADKISWKITDPVWVDQWPLTYEKTAAIALVQEQLAAGHIEPTNSPWNTPIFIKKKSGSWRLLQDLRAVNKVMVPMGALQPGPLSPVAIPLNYHKIVIDLKDCFFTIPLHPEDRPYFAFSVPQINFQSPMPRYQWKVLPQGMANSPTLCQKFVAAAIAPVRSQWPEAYILHYMDILLACDSA EAAKACYAHIISCLTSYGLKIAPDKVQVSEPFYSLGFELHHQVFTPRVCLKTDHLKTLNDFQKLLGDIQWLRPYLKLPTSAVLPLNLIKGDPNPLSV RALTPKAEKQSLALINKAIQNQSVQGISYNLPLVLLLTPTHTPTAVFWQPNGTDPKNGSPLLWLHLPASPSKVLLTYPSSLAMLIIKGRYTRGQLFGRDPHSIIIFYTQDQLTWLLQTSDEWAIALSSFTGDIDNHYSPDPVIAQFALKHQFIFPKITKCAPIQATLVFTDGSNGIAAYVIDNQPSIKSPYLSAQLVE LYAILQVFTVLAHQPFNLTYDSAYIAQSVPLLETVPFIKSSSTNATPLFSKLQQLILNRQHPFFIGHLRAHLNLPGLPACGNALADAATQIFPIISD
SRV2_P51517	LATAVDILAPQRYADPITWKSDEPWWVDQWPLTQEKLAAQQLVQEQLAAGHIESNSPWNTPIFVKKKSGKWRLQDLRAVNATMVLGALQPGLPSPVAIPQGYFKIVIDLKDCFFTIPLQPVQDKRFASFSLPSTNFKQPMKRYQWKVLPQGMANSPTLCQKYVAAAIAPVRKSWAQMYIIHYMDILLIAG KLGEQVLQCFQALKQALTTTGLQIAPEKVQLQDPYTYLGFQINGPKITNQKAVIRRDKLQTLNDFQKLLGDIQWLRPYLHLTTGDLKPLFDILKGDSPNSPRSLSEALASLQKVEIAIEQFVTQIDYTQPLTFLIFNTLTPTGLFWQNNPVMVWHLASPCKVLLPYDAIADLILGRDNSKKYFGLPESTII QPYYSKSIHWMQNTETWPIACASYAGNIDNHYPPNKLQFCKLHVVFPRIISKTPLDNALLVFTDGSSTGIAAYTEKTTVRFKTSHTSAQLVELQA LIAVLAFPHRALNVYTD SAYLAHSIPLLETVSHIKHISDTAKFFLQCCQLLYNRSIFPYLGHIRAHSGLPGLSQGNHITDLATKVVATLTT
SRV2_P51517_2mutB	LATAVDILAPQRYADPITWKSDEPWWVDQWPLTQEKLAAQQLVQEQLAAGHIESNSPWNTPIFVKKKSGKWRLQDLRAVNATMVLGALQPGLPSPVAIPQGYFKIVIDLKDCFFTIPLQPVQDKRFASFSLPSTNFKQPMKRYQWKVLPQGMANSPTLCQKYVAAAIAPVRKSWAQMYIIHYMDILLIAG KLGEQVLQCFQALKQALTTTGLQIAPEKVQLQDPYTYLGFQINGPKITNQKAVIRRDKLQTLNDFQKLLGDIQWLRPYLHLTTGDLKPLFDILKGDSPNSPRSLSEALASLQKVEIAIEQFVTQIDYTQPLTFLIFNTLTPTGLFWQNNPVMVWHLASPCKVLLPYDAIADLILGRDNSKKYFGLPESTII QPYYSKSIHWMQNTETWPIACASYAGNIDNHYPPNKLQFCKLHVVFPRIISKTPLDNALLVFTDGSSTGIAAYTEKTTVRFKTSHTSAQLVELQA LIAVLAFPHRALNVYTD SAYLAHSIPLLETVSHIKHISDTAKFFLQCCQLLYNRSIFPYLGHIRAHSGLPGLSQGNHITDLATKVVATLTT
WDSV_O92815	SCQTNTLNIDEYLLQFPDQLWASLPTDGRMLVPPITIKIDNASLSIRQYLPKDKTEGLRPLISSLENQGLIKCHSPCNTPIFPIKAGRDEYRMI HDLRAINNIVAPLTAVVASPTTVLSNLAPSLHWFTVIDLSNAFFSVPIHKDSQYLAFTFEHGYQYTWTVLPQGFHSPTLFSQALYQSLHKIKFISSEIC IYMDVLIASKDRDNTNLDKTAVMLGHLASEGHKVSKKLQLCCQEVVYLGQLLTPGKRILPDRKVTVSQFQPTTIRQIRAFGLGVGCRHWIPEF SIHSEKLEKQLKDTAEFPQLDDQQVEAFNKLKHAITAPVLPVDPAPKPFQLYTSHSEHASIAVLTKQHAGRTRPIAFLSSKFDAIESGLPCLKACA SIHRSLTQADSFILGAPLIITYTHAICTLLQRDRSQLVTASRFSKWEADLLRPELTFVACSAVSPAHLVMSQSCENNIPPHDCVLLTHTISRPRPDLSDLP

RT Name	RT amino acid sequence
	IPDPMTLFSDGSYTTGRGGAAVVMHRPVTDDFIIHQPGGASQAETALLAAACHLATDKTVNIYTDTRYAYGVVHDFGHLWMHGRFVTSAGT PIKNHKEIEYLLKQIMPKQVSVIKIEAHTKGVSMVEVRGNAADAAKNAVFLVQR
WDSV_092815_2mut	SCQTKNTLNIDEYLLQFPDQLWASLPTDIGRMLVPPITIKIKDNASLPSIRQYPLPKDKTEGLRPLISSLENQGILKCHSPCNPITPIFKKAGRDEYRMI HDLRAINIVAPLTAVVASPTTVLSNLAPSLHWFTVIDLSNAFFSVPIHKDSQYLFATFEFGHQYTWTVLPQGFIHSPITLNFQALYQSLHKIKFKISSEIC IYMDLVLIASQRDRDNLKDTAVMLQHLASEGHKVSKKLQLCQEQVYVYLGQLLTPEGRKILPDRKVTVSQFQQPTTIRQIRAFGLGVGYCRHWPFEF SIHSKFLEKQLKPDTAEPFQLDDQVQVAFNKLKHAITAPVLVVPDPAKPFQLYTSHSEHASIAVLTQKHAGRTRPIAFLSSKFDAIESGLPPLKACA SIHRSLTQADSFILGAPLIYTTTHAICTLLQRDRSQLVTSRFSKWEADLLRPELTFVACSAVSPAHLVMQSCENNIPPHDCVLLTHTISRPPDLSLPI IPDPMTLFSDGSYTTGRGGAAVVMHRPVTDDFIIHQPGGASQAETALLAAACHLATDKTVNIYTDTRYAYGVVHDFGHLWMHGRFVTSAGT PIKNHKEIEYLLKQIMPKQVSVIKIEAHTKGVSMVEVRGNAADAAKNAVFLVQR
WDSV_092815_2mutA	SCQTKNTLNIDEYLLQFPDQLWASLPTDIGRMLVPPITIKIKDNASLPSIRQYPLPKDKTEGLRPLISSLENQGILKCHSPCNPITPIFKKAGRDEYRMI HDLRAINIVAPLTAVVASPTTVLSNLAPSLHWFTVIDLSNAFFSVPIHKDSQYLFATFEFGHQYTWTVLPQGFIHSPITLNFQALYQSLHKIKFKISSEIC IYMDLVLIASQRDRDNLKDTAVMLQHLASEGHKVSKKLQLCQEQVYVYLGQLLTPEGRKILPDRKVTVSQFQQPTTIRQIRAFGLGVGYCRHWPFEF IHSKFLEKQLKPDTAEPFQLDDQVQVAFNKLKHAITAPVLVVPDPAKPFQLYTSHSEHASIAVLTQKHAGRTRPIAFLSSKFDAIESGLPPLKACASI RRSLTQADSFILGAPLIYTTTHAICTLLQRDRSQLVTSRFSKWEADLLRPELTFVACSAVSPAHLVMQSCENNIPPHDCVLLTHTISRPPDLSLPI DPDMLTSDGSYTTGRGGAAVVMHRPVTDDFIIHQPGGASQAETALLAAACHLATDKTVNIYTDTRYAYGVVHDFGHLWMHGRFVTSAGTPI KNHKEIEYLLKQIMPKQVSVIKIEAHTKGVSMVEVRGNAADAAKNAVFLVQR
WMSV_P0335_9	VLNLEEEYRLHEKVPVPSIDPSWLQLFPTVWAERAGMGLANQVPPVVVELRSGASPVAVRQYPMSEAREGIRPHIQRFDLGLVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFDEALHRDLAPFRALNPQVLLQYVDDLVAAPTYRDCKEGQKLLQELSKLGYRVSAKKAQLCQKEVTVLYGLLKEGKRWLTAPARKATVM KIPPTTPRQVREFLGAGFCRLWIPGFAASLAAPLYLTKESIPFIWTEEHQKAFDRKEALLSAPALAPDLTKPFTLYVDERAGVARGVLTQTLGPW RRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKADAKLTLGQNVTVIASHLESIVRQPPDRWMTNARMTHYQSLNERNVSPAPPVNLNATLLPV ESEATPVHRCSELAETGTTRDLKDQPLPGVPAWYTDGSSFIAEGKRRAGAAVVGKRTVWASSLPEGTSQAELVALTQALRAEGKINDIYTD SRYAFATAHIGAIYKQRGWLTSGAKDIKNKEILALLEAIHLPKRVAIHCPGHQKGNPDVATGNRRADEAAKQAALSTRVLAETTKP
WMSV_P0335_9_3mut	VLNLEEEYRLHEKVPVPSIDPSWLQLFPTVWAERAGMGLANQVPPVVVELRSGASPVAVRQYPMSEAREGIRPHIQRFDLGLVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLAPFRALNPQVLLQYVDDLVAAPTYRDCKEGQKLLQELSKLGYRVSAKKAQLCQKEVTVLYGLLKEGKRWLTAPARKATVM KIPPTTPRQVREFLGAGFCRLWIPGFAASLAAPLYLTKPSIPFIWTEEHQKAFDRKEALLSAPALAPDLTKPFTLYVDERAGVARGVLTQTLGPW RRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKADAKLTLGQNVTVIASHLESIVRQPPDRWMTNARMTHYQSLNERNVSPAPPVNLNATLLPV ESEATPVHRCSELAETGTTRDLKDQPLPGVPAWYTDGSSFIAEGKRRAGAAVVGKRTVWASSLPEGTSQAELVALTQALRAEGKINDIYTD SRYAFATAHIGAIYKQRGWLTSGAKDIKNKEILALLEAIHLPKRVAIHCPGHQKGNPDVATGNRRADEAAKQAALSTRVLAETTKP
WMSV_P0335_9_3mutA	VLNLEEEYRLHEKVPVPSIDPSWLQLFPTVWAERAGMGLANQVPPVVVELRSGASPVAVRQYPMSEAREGIRPHIQRFDLGLVPCQSPWNTPL LLPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLAPFRALNPQVLLQYVDDLVAAPTYRDCKEGQKLLQELSKLGYRVSAKKAQLCQKEVTVLYGLLKEGKRWLTAPARKATVM KIPPTTPRQVREFLGAGFCRLWIPGFAASLAAPLYLTKPSIPFIWTEEHQKAFDRKEALLSAPALAPDLTKPFTLYVDERAGVARGVLTQTLGPW RRPVAYLSKKLDPVAGSWPTCLKAVAAVALLKADAKLTLGQNVTVIASHLESIVRQPPDRWMTNARMTHYQSLNERNVSPAPPVNLNATLLPV ESEATPVHRCSELAETGTTRDLKDQPLPGVPAWYTDGSSFIAEGKRRAGAAVVGKRTVWASSLPEGTSQAELVALTQALRAEGKINDIYTD SRYAFATAHIGAIYKQRGWLTSGAKDIKNKEILALLEAIHLPKRVAIHCPGHQKGNPDVATGNRRADEAAKQAALSTRVLAETTKP
XMRV6_A1265_1	TLNIEDEYRLHETSKEDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIKQYPMSEARLGIKPHIQRLDDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFDEALHRDLADFRQHPLDILLQYVDDLAAATSEQDCQGRTRALLQTLGNLGYRASAKKAQICQKQVYLYGLLKEGQRWLTAPARKATVM GQPTPKTPRQLREFLGAGFCRLWIPGFAEMAAPLYLTKPTGTLFNWGPDPQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQL GPWRRPVAYLSKKLDPVAGSWPTCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQFGPVVALN PATLLPLPEKEAPHDCLEIAETHGTRPDLTQPIPDADYTWYTDGSSFLQEGQRRAGAAVTETETIWARALPAGTSAQRAELIALTQALKMAEGK KLNVTYDSRYAFATAHIGAIYRRRGWLTSEGREIKNKNEILALLKALFLPKRLSIHCPGHQKGNSEARGNRMADQAAREAAAMKAVLETSTLL
XMRV6_A1265_1_3mut	TLNIEDEYRLHETSKEDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIKQYPMSEARLGIKPHIQRLDDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLADFRQHPLDILLQYVDDLAAATSEQDCQGRTRALLQTLGNLGYRASAKKAQICQKQVYLYGLLKEGQRWLTAPARKATVM GQPTPKTPRQLREFLGAGFCRLWIPGFAEMAAPLYLTKPTGTLFNWGPDPQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQL GPWRRPVAYLSKKLDPVAGSWPTCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQFGPVVALN PATLLPLPEKEAPHDCLEIAETHGTRPDLTQPIPDADYTWYTDGSSFLQEGQRRAGAAVTETETIWARALPAGTSAQRAELIALTQALKMAEGK KLNVTYDSRYAFATAHIGAIYRRRGWLTSEGREIKNKNEILALLKALFLPKRLSIHCPGHQKGNSEARGNRMADQAAREAAAMKAVLETSTLL
XMRV6_A1265_1_3mutA	TLNIEDEYRLHETSKEDVPLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSIKQYPMSEARLGIKPHIQRLDDQGILVPCQSPWNTPL LPVKKPGTNDYRPVQDLREINKRVQDIHPTVNPYNLLSGLPPSHQWYTVLDLKDFAFFCLRLHPTSQPLFAFEWRDPEMGISGQLTWTRLPQGFK NSPTLFNEALHRDLADFRQHPLDILLQYVDDLAAATSEQDCQGRTRALLQTLGNLGYRASAKKAQICQKQVYLYGLLKEGQRWLTAPARKATVM GQPTPKTPRQLREFLGAGFCRLWIPGFAEMAAPLYLTKPTGTLFNWGPDPQKAYQEIQALLTAPALGLPDLTKPFELFVDEKQGYAKGVLTKQL GPWRRPVAYLSKKLDPVAGSWPTCLRMVAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQAMLLDTRVQFGPVVALN PATLLPLPEKEAPHDCLEIAETHGTRPDLTQPIPDADYTWYTDGSSFLQEGQRRAGAAVTETETIWARALPAGTSAQRAELIALTQALKMAEGK KLNVTYDSRYAFATAHIGAIYRRRGWLTSEGREIKNKNEILALLKALFLPKRLSIHCPGHQKGNSEARGNRMADQAAREAAAMKAVLETSTLL

In some embodiments, reverse transcriptase domains are modified, for example by site-specific mutation. In some embodiments, reverse transcriptase domains are engineered to have improved properties, e.g. SuperScript IV (SSIV) reverse transcriptase derived from the MMLV

- RT. In some embodiments, the reverse transcriptase domain may be engineered to have lower error rates, e.g., as described in WO2001068895, incorporated herein by reference. In some embodiments, the reverse transcriptase domain may be engineered to be more thermostable. In some embodiments, the reverse transcriptase domain may be engineered to be more processive.
- 5 In some embodiments, the reverse transcriptase domain may be engineered to have tolerance to inhibitors. In some embodiments, the reverse transcriptase domain may be engineered to be faster. In some embodiments, the reverse transcriptase domain may be engineered to better tolerate modified nucleotides in the RNA template. In some embodiments, the reverse transcriptase domain may be engineered to insert modified DNA nucleotides. In some
- 10 embodiments, the reverse transcriptase domain is engineered to bind a template RNA. In some embodiments, one or more mutations are chosen from D200N, L603W, T330P, D524G, E562Q, D583N, P51L, S67R, E67K, T197A, H204R, E302K, F309N, W313F, L435G, N454K, H594Q, L671P, E69K, or D653N in the RT domain of murine leukemia virus reverse transcriptase or a corresponding mutation at a corresponding position of another RT domain.
- 15 In some embodiments, a gene modifying polypeptide comprises the RT domain from a retroviral reverse transcriptase, e.g., a wild-type M-MLV RT, e.g., comprising the following sequence:
- M-MLV (WT):*
- TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVS
- 20 KQYPMSQEARLGKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREVNK
RVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGIS
GQLTWTRL PQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCCQG
TRALLQTLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAP
- 25 ALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRM
VAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLD TDR
VQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSL
LQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLVNVTDSRY
AFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEAR
- 30 GNRMADQAARKAAITETPDTSTLLI (SEQ ID NO: 4012)

In some embodiments, a gene modifying polypeptide comprises the RT domain from a retroviral reverse transcriptase, e.g., an M-MLV RT, e.g., comprising the following sequence:

TLNIEDEHRLHETSKPEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVS
 KQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREV
 5 RVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGIS
 GQLTWTRL PQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCCQG
 TRALLQTLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWLTEARKETVMGQPTPKT
 PRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAYQEIKQALLTAP
 ALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPVAAGWPPCLRM
 10 VAAIAVLTKDAGKLTMGQPLVILAPHA VEALVKQPPDRWLSNARMTHYQALLLDTDR
 VQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDADHTWYTDGSSL
 LQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLVYTD SRY
 AFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEAR
 GNRMADQAARKAAITETPDTSTLL (SEQ ID NO: 4013)

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In some embodiments, a gene modifying polypeptide comprises the RT domain from a retroviral reverse transcriptase comprising the sequence of amino acids 659-1329 of NP_057933. In embodiments, the gene modifying polypeptide further comprises one additional amino acid at the N-terminus of the sequence of amino acids 659-1329 of NP_057933, e.g., as shown below:

TLNIEDEHRLHETSKPEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVS
 KQYPMSQEARLGIKPHIQRLLDQGILVPCQSPWNTPLL PVKKPGTNDYRPVQDLREV
 20 **KRVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDP**
EMGISGQLTWTRL PQGFKNSPTLFDEALHRDLADFRIQHPDLILLQYVDDLLLAAT
SELDCCQGTRALLQTLGNLGYRASAKKAQICQKQVKYLG YLLKEGQRWLTEARKE
 25 TVMGQPTPKTPRQLREFLGTAGFCRLWIPGFAEMAAPLYPLTKTGTLFNWGPDQQKAY
 QEIKQALLTAPALGLPDLTKPFELFVDEKQGYAKGVL TQKLGPWRRPVAYLSKKLDPV
 AAGWPPCLRMVAAIAVLTKDAGKLTMGQPLVILAPHA VEALVKQPPDRWLSNARMTH
 YQALLLDTDRVQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPDAD
HTWYTDGSSLLQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGK
 30 KLNVYTD SRYAFATAHIHGEIYRRRGLLTSEGKEIKNKDEILALLKALFLPKRLSIHCPG
HQKGHSAEARGNRMADQAARKAA (SEQ ID NO: 4014)

Core RT (bold), annotated per above

RNAseH (underlined), annotated per above

35

In embodiments, the gene modifying polypeptide further comprises one additional amino acid at the C-terminus of the sequence of amino acids 659-1329 of NP_057933. In embodiments, the gene modifying polypeptide comprises an RNaseH1 domain (e.g., amino acids 1178-1318 of NP_057933).

5 In some embodiments, a retroviral reverse transcriptase domain, e.g., M-MLV RT, may comprise one or more mutations from a wild-type sequence that may improve features of the RT, e.g., thermostability, processivity, and/or template binding. In some embodiments, an M-MLV RT domain comprises, relative to the M-MLV (WT) sequence above, one or more mutations, e.g., selected from D200N, L603W, T330P, T306K, W313F, D524G, E562Q, D583N, P51L,
10 S67R, E67K, T197A, H204R, E302K, F309N, L435G, N454K, H594Q, D653N, R110S, K103L, e.g., a combination of mutations, such as D200N, L603W, and T330P, optionally further including T306K and W313F. In some embodiments, an M-MLV RT used herein comprises the mutations D200N, L603W, T330P, T306K and W313F. In embodiments, the mutant M-MLV RT comprises the following amino acid sequence:

15

M-MLV (PE2):

TLNIEDEYRLHETSKEPDVSLGSTWLSDFPQAWAETGGMGLAVRQAPLIPLKATSTPVSI
KQYPMSQEARLGKPHIQRLLDQGILVPCQSPWNTPLLVPKKPGTNDYRPVQDLREVNK
RVEDIHPTVPNPYNLLSGLPPSHQWYTVLDLKDAFFCLRLHPTSQPLFAFEWRDPEMGIS
20 GQLTWTRLPPQGFKNSPTLFNEALHRDLADFRIQHPDLILLQYVDDLLLAATSELDCQQG
TRALLQTLGNLGYRASAKKAQICQKQVKYLGILLKEGQRWLTEARKETVMGQPTPKT
PRQLREFLGKAGFCRLFIPGFAEMAAPLYPLTKPGTLFNWGPDQQKAYQEIKQALLTAP
ALGLPDLTKPFELFVDEKQGYAKGVLTQKLGPWRRPVAYLSKKLDPVAAGWPPCLRM
VAAIAVLTKDAGKLTMGQPLVILAPHAVEALVKQPPDRWLSNARMTHYQALLLDTDR
25 VQFGPVVALNPATLLPLPEEGLQHNCLDILAEAHGTRPDLTDQPLPADHTWYTDGSSL
LQEGQRKAGAAVTTETEVIWAKALPAGTSAQRAELIALTQALKMAEGKKLVNVTDSRY
AFATAHIHGEIYRRRGWLTSEGKEIKNKDEILALLKALFLPKRLSIHCPGHQKGHSAEAR
GNRMADQAARKAAITETPDTSTLLI (SEQ ID NO: 4015)

In some embodiments, a writing domain (e.g., RT domain) comprises an RNA-binding
30 domain, e.g., that specifically binds to an RNA sequence. In some embodiments, a template

RNA comprises an RNA sequence that is specifically bound by the RNA-binding domain of the writing domain.

In some embodiments, the reverse transcription domain only recognizes and reverse transcribes a specific template, e.g., a template RNA of the system. In some embodiments, the template comprises a sequence or structure that enables recognition and reverse transcription by a reverse transcription domain. In some embodiments, the template comprises a sequence or structure that enables association with an RNA-binding domain of a polypeptide component of a genome engineering system described herein. In some embodiments, the genome engineering system reverse preferably transcribes a template comprising an association sequence over a template lacking an association sequence.

The writing domain may also comprise DNA-dependent DNA polymerase activity, e.g., comprise enzymatic activity capable of writing DNA into the genome from a template DNA sequence. In some embodiments, DNA-dependent DNA polymerization is employed to complete second-strand synthesis of a target site edit. In some embodiments, the DNA-dependent DNA polymerase activity is provided by a DNA polymerase domain in the polypeptide. In some embodiments, the DNA-dependent DNA polymerase activity is provided by a reverse transcriptase domain that is also capable of DNA-dependent DNA polymerization, e.g., second-strand synthesis. In some embodiments, the DNA-dependent DNA polymerase activity is provided by a second polypeptide of the system. In some embodiments, the DNA-dependent DNA polymerase activity is provided by an endogenous host cell polymerase that is optionally recruited to the target site by a component of the genome engineering system.

In some embodiments, the reverse transcriptase domain has a lower probability of premature termination rate (P_{off}) *in vitro* relative to a reference reverse transcriptase domain. In some embodiments, the reference reverse transcriptase domain is a viral reverse transcriptase domain, e.g., the RT domain from M-MLV.

In some embodiments, the reverse transcriptase domain has a lower probability of premature termination rate (P_{off}) *in vitro* of less than about $5 \times 10^{-3}/\text{nt}$, $5 \times 10^{-4}/\text{nt}$, or $5 \times 10^{-6}/\text{nt}$, e.g., as measured on a 1094 nt RNA. In embodiments, the *in vitro* premature termination rate is determined as described in Bibillo and Eickbush (2002) J Biol Chem 277(38):34836-34845 (incorporated by reference herein its entirety).

In some embodiments, the reverse transcriptase domain is able to complete at least about 30% or 50% of integrations in cells. The percent of complete integrations can be measured by dividing the number of substantially full-length integration events (e.g., genomic sites that comprise at least 98% of the expected integrated sequence) by the number of total (including substantially full-length and partial) integration events in a population of cells. In embodiments, the integrations in cells is determined (e.g., across the integration site) using long-read amplicon sequencing, e.g., as described in Karst et al. (2020) *bioRxiv* doi.org/10.1101/645903 (incorporated by reference herein in its entirety).

In embodiments, quantifying integrations in cells comprises counting the fraction of integrations that contain at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the DNA sequence corresponding to the template RNA (e.g., a template RNA having a length of at least 0.05, 0.1, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 3, 4, or 5 kb, e.g., a length between 0.5-0.6, 0.6-0.7, 0.7-0.8, 0.8-0.9, 1.0-1.2, 1.2-1.4, 1.4-1.6, 1.6-1.8, 1.8-2.0, 2-3, 3-4, or 4-5 kb).

In some embodiments, the reverse transcriptase domain is capable of polymerizing dNTPs *in vitro*. In embodiments, the reverse transcriptase domain is capable of polymerizing dNTPs *in vitro* at a rate between 0.1 – 50 nt/sec (e.g., between 0.1-1, 1-10, or 10-50 nt/sec). In embodiments, polymerization of dNTPs by the reverse transcriptase domain is measured by a single-molecule assay, e.g., as described in Schwartz and Quake (2009) *PNAS* 106(48):20294-20299 (incorporated by reference in its entirety).

In some embodiments, the reverse transcriptase domain has an *in vitro* error rate (e.g., misincorporation of nucleotides) of between $1 \times 10^{-3} - 1 \times 10^{-4}$ or $1 \times 10^{-4} - 1 \times 10^{-5}$ substitutions/nt, e.g., as described in Yasukawa et al. (2017) *Biochem Biophys Res Commun* 492(2):147-153 (incorporated herein by reference in its entirety). In some embodiments, the reverse transcriptase domain has an error rate (e.g., misincorporation of nucleotides) in cells (e.g., HEK293T cells) of between $1 \times 10^{-3} - 1 \times 10^{-4}$ or $1 \times 10^{-4} - 1 \times 10^{-5}$ substitutions/nt, e.g., by long-read amplicon sequencing, e.g., as described in Karst et al. (2020) *bioRxiv* doi.org/10.1101/645903 (incorporated by reference herein in its entirety).

In some embodiments, the reverse transcriptase domain is capable of performing reverse transcription of a target RNA *in vitro*. In some embodiments, the reverse transcriptase requires a primer of at least 3 nucleotides to initiate reverse transcription of a template. In some embodiments, reverse transcription of the target RNA is determined by detection of cDNA from

the target RNA (e.g., when provided with a ssDNA primer, e.g., which anneals to the target with at least 3, 4, 5, 6, 7, 8, 9, or 10 nt at the 3' end), e.g., as described in Bibillo and Eickbush (2002) *J Biol Chem* 277(38):34836-34845 (incorporated herein by reference in its entirety).

In some embodiments, the reverse transcriptase domain performs reverse transcription at least 5 or 10 times more efficiently (e.g., by cDNA production), e.g., when converting its RNA template to cDNA, for example, as compared to an RNA template lacking the protein binding motif (e.g., a 3' UTR). In embodiments, efficiency of reverse transcription is measured as described in Yasukawa et al. (2017) *Biochem Biophys Res Commun* 492(2):147-153 (incorporated by reference herein in its entirety).

In some embodiments, the reverse transcriptase domain specifically binds a specific RNA template with higher frequency (e.g., about 5 or 10-fold higher frequency) than any endogenous cellular RNA, e.g., when expressed in cells (e.g., HEK293T cells). In embodiments, frequency of specific binding between the reverse transcriptase domain and the template RNA are measured by CLIP-seq, e.g., as described in Lin and Miles (2019) *Nucleic Acids Res* 47(11):5490-5501 (incorporated herein by reference in its entirety).

Template nucleic acid binding domain

The gene modifying polypeptide typically contains regions capable of associating with the template nucleic acid (e.g., template RNA). In some embodiments, the template nucleic acid binding domain is an RNA binding domain. In some embodiments, the RNA binding domain is a modular domain that can associate with RNA molecules containing specific signatures, e.g., structural motifs. In other embodiments, the template nucleic acid binding domain (e.g., RNA binding domain) is contained within the reverse transcription domain, e.g., the reverse transcriptase-derived component has a known signature for RNA preference.

In other embodiments, the template nucleic acid binding domain (e.g., RNA binding domain) is contained within the target DNA binding domain. For example, in some embodiments, the DNA binding domain is a CRISPR-associated protein that recognizes the structure of a template nucleic acid (e.g., template RNA) comprising a gRNA. In some embodiments, a gene modifying polypeptide comprises a DNA-binding domain comprising a CRISPR-associated protein that associates with a gRNA scaffold that allows the DNA-binding domain to bind a target genomic DNA sequence. In some embodiments, the gRNA scaffold and

gRNA spacer is comprised within the template nucleic acid (e.g., template RNA), thus the DNA-binding domain is also the template nucleic acid binding domain. In some embodiments, the polypeptide possesses RNA binding function in multiple domains, e.g., can bind a gRNA structure in a CRISPR-associated DNA binding domain and an additional sequence or structure
 5 in a reverse transcriptase domain.

In some embodiments, the RNA binding domain is capable of binding to a template RNA with greater affinity than a reference RNA binding domain. In some embodiments, the reference RNA binding domain is an RNA binding domain from Cas9 of *S. pyogenes*. In some
 10 embodiments, the RNA binding domain is capable of binding to a template RNA with an affinity between 100 pM – 10 nM (e.g., between 100 pM-1 nM or 1 nM – 10 nM). In some
 embodiments, the affinity of an RNA binding domain for its template RNA is measured *in vitro*, e.g., by thermophoresis, e.g., as described in Asmari et al. Methods 146:107-119 (2018)
 (incorporated by reference herein in its entirety). In some embodiments, the affinity of an RNA
 binding domain for its template RNA is measured in cells (e.g., by FRET or CLIP-Seq).

15 In some embodiments, the RNA binding domain is associated with the template RNA *in vitro* at a frequency at least about 5-fold or 10-fold higher than with a scrambled RNA. In some
 embodiments, the frequency of association between the RNA binding domain and the template RNA or scrambled RNA is measured by CLIP-seq, e.g., as described in Lin and Miles (2019)
Nucleic Acids Res 47(11):5490-5501 (incorporated by reference herein in its entirety). In some
 20 embodiments, the RNA binding domain is associated with the template RNA in cells (e.g., in HEK293T cells) at a frequency at least about 5-fold or 10-fold higher than with a scrambled
 RNA. In some embodiments, the frequency of association between the RNA binding domain and the template RNA or scrambled RNA is measured by CLIP-seq, e.g., as described in Lin and
 Miles (2019), *supra*.

25 *Endonuclease domains and DNA binding domains*

In some embodiments, a gene modifying polypeptide possesses the function of DNA target site cleavage via an endonuclease domain. In some embodiments, a gene modifying polypeptide comprises a DNA binding domain, e.g., for binding to a target nucleic acid. In some
 30 embodiments, a domain (e.g., a Cas domain) of the gene modifying polypeptide comprises two or more smaller domains, e.g., a DNA binding domain and an endonuclease domain. It is

understood that when a DNA binding domain (e.g., a Cas domain) is said to bind to a target nucleic acid sequence, in some embodiments, the binding is mediated by a gRNA.

In some embodiments, a domain has two functions. For example, in some embodiments, the endonuclease domain is also a DNA-binding domain. In some embodiments, the
5 endonuclease domain is also a template nucleic acid (e.g., template RNA) binding domain. For example, in some embodiments, a polypeptide comprises a CRISPR-associated endonuclease domain that binds a template RNA comprising a gRNA, binds a target DNA sequence (e.g., with complementarity to a portion of the gRNA), and cuts the target DNA sequence. In some
10 embodiments, an endonuclease domain or endonuclease/DNA-binding domain from a heterologous source can be used or can be modified (e.g., by insertion, deletion, or substitution of one or more residues) in a gene modifying system described herein.

In some embodiments, a nucleic acid encoding the endonuclease domain or endonuclease/DNA binding domain is altered from its natural sequence to have altered codon usage, e.g. improved for human cells. In some embodiments, the endonuclease element is a
15 heterologous endonuclease element, such as a Cas endonuclease (e.g., Cas9), a type-II restriction endonuclease (e.g., FokI), a meganuclease (e.g., I-SceI), or other endonuclease domain.

In certain aspects, the DNA-binding domain of a gene modifying polypeptide described herein is selected, designed, or constructed for binding to a desired host DNA target sequence. In certain embodiments, the DNA-binding domain of the polypeptide is a heterologous DNA-
20 binding element. In some embodiments the heterologous DNA binding element is a zinc-finger element or a TAL effector element, e.g., a zinc-finger or TAL polypeptide or functional fragment thereof. In some embodiments the heterologous DNA binding element is a sequence-guided DNA binding element, such as Cas9, Cpf1, or other CRISPR-related protein that has been altered to have no endonuclease activity. In some embodiments the heterologous DNA binding element
25 retains endonuclease activity. In some embodiments, the heterologous DNA binding element retains partial endonuclease activity to cleave ssDNA, e.g., possesses nickase activity. In specific embodiments, the heterologous DNA-binding domain can be any one or more of Cas9, TAL domain, ZF domain, Myb domain, combinations thereof, or multiples thereof.

In some embodiments, DNA-binding domains are modified, for example by site-specific
30 mutation, increasing or decreasing DNA-binding elements (for example, number and/or specificity of zinc fingers), etc., to alter DNA-binding specificity and affinity. In some

embodiments a nucleic acid sequence encoding the DNA binding domain is altered from its natural sequence to have altered codon usage, e.g. improved for human cells. In embodiments, the DNA binding domain comprises one or more modifications relative to a wild-type DNA binding domain, e.g., a modification via directed evolution, e.g., phage-assisted continuous evolution (PACE).

In some embodiments, the DNA binding domain comprises a meganuclease domain (e.g., as described herein, e.g., in the endonuclease domain section), or a functional fragment thereof. In some embodiments, the meganuclease domain possesses endonuclease activity, e.g., double-strand cleavage and/or nickase activity. In other embodiments, the meganuclease domain has reduced activity, e.g., lacks endonuclease activity, e.g., the meganuclease is catalytically inactive. In some embodiments, a catalytically inactive meganuclease is used as a DNA binding domain, e.g., as described in Fonfara et al. *Nucleic Acids Res* 40(2):847-860 (2012), incorporated herein by reference in its entirety.

In some embodiments, a gene modifying polypeptide comprises a modification to a DNA-binding domain, e.g., relative to the wild-type polypeptide. In some embodiments, the DNA-binding domain comprises an addition, deletion, replacement, or modification to the amino acid sequence of the original DNA-binding domain. In some embodiments, the DNA-binding domain is modified to include a heterologous functional domain that binds specifically to a target nucleic acid (e.g., DNA) sequence of interest. In some embodiments, the functional domain replaces at least a portion (e.g., the entirety of) the prior DNA-binding domain of the polypeptide. In some embodiments, the functional domain comprises a zinc finger (e.g., a zinc finger that specifically binds to the target nucleic acid (e.g., DNA) sequence of interest. In some embodiments, the functional domain comprises a Cas domain (e.g., a Cas domain that specifically binds to the target nucleic acid (e.g., DNA) sequence of interest. In some embodiments, the Cas domain comprises a Cas9 or a mutant or variant thereof (e.g., as described herein). In embodiments, the Cas domain is associated with a guide RNA (gRNA), e.g., as described herein. In embodiments, the Cas domain is directed to a target nucleic acid (e.g., DNA) sequence of interest by the gRNA. In embodiments, the Cas domain is encoded in the same nucleic acid (e.g., RNA) molecule as the gRNA. In embodiments, the Cas domain is encoded in a different nucleic acid (e.g., RNA) molecule from the gRNA.

In some embodiments, the DNA binding domain is capable of binding to a target sequence (e.g., a dsDNA target sequence) with greater affinity than a reference DNA binding domain. In some embodiments, the reference DNA binding domain is a DNA binding domain from Cas9 of *S. pyogenes*. In some embodiments, the DNA binding domain is capable of binding to a target sequence (e.g., a dsDNA target sequence) with an affinity between 100 pM – 10 nM (e.g., between 100 pM-1 nM or 1 nM – 10 nM).

In some embodiments, the affinity of a DNA binding domain for its target sequence (e.g., dsDNA target sequence) is measured in vitro, e.g., by thermophoresis, e.g., as described in Asmari et al. *Methods* 146:107-119 (2018) (incorporated by reference herein in its entirety).

In embodiments, the DNA binding domain is capable of binding to its target sequence (e.g., dsDNA target sequence), e.g., with an affinity between 100 pM – 10 nM (e.g., between 100 pM-1 nM or 1 nM – 10 nM) in the presence of a molar excess of scrambled sequence competitor dsDNA, e.g., of about 100-fold molar excess.

In some embodiments, the DNA binding domain is found associated with its target sequence (e.g., dsDNA target sequence) more frequently than any other sequence in the genome of a target cell, e.g., human target cell, e.g., as measured by ChIP-seq (e.g., in HEK293T cells), e.g., as described in He and Pu (2010) *Curr. Protoc Mol Biol* Chapter 21 (incorporated herein by reference in its entirety). In some embodiments, the DNA binding domain is found associated with its target sequence (e.g., dsDNA target sequence) at least about 5-fold or 10-fold, more frequently than any other sequence in the genome of a target cell, e.g., as measured by ChIP-seq (e.g., in HEK293T cells), e.g., as described in He and Pu (2010), *supra*.

In some embodiments, the endonuclease domain has nickase activity and cleaves one strand of a target DNA. In some embodiments, nickase activity reduces the formation of double-stranded breaks at the target site. In some embodiments, the endonuclease domain creates a staggered nick structure in the first and second strands of a target DNA. In some embodiments, a staggered nick structure generates free 3' overhangs at the target site. In some embodiments, free 3' overhangs at the target site improve editing efficiency, e.g., by enhancing access and annealing of a 3' homology region of a template nucleic acid. In some embodiments, a staggered nick structure reduces the formation of double-stranded breaks at the target site.

In some embodiments, the endonuclease domain cleaves both strands of a target DNA, e.g., results in blunt-end cleavage of a target with no ssDNA overhangs on either side of the cut-

site. The amino acid sequence of an endonuclease domain of a gene modifying system described herein may be at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% identical to the amino acid sequence of an endonuclease domain described herein.

In certain embodiments, the heterologous endonuclease is FokI or a functional fragment thereof. In certain embodiments, the heterologous endonuclease is a Holliday junction resolvase or homolog thereof, such as the Holliday junction resolving enzyme from *Sulfolobus solfataricus*—SsoI Hje (Govindaraju et al., *Nucleic Acids Research* 44:7, 2016). In certain embodiments, the heterologous endonuclease is the endonuclease of the large fragment of a spliceosomal protein, such as Prp8 (Mahbub et al., *Mobile DNA* 8:16, 2017). In certain embodiments, the heterologous endonuclease is derived from a CRISPR-associated protein, e.g., Cas9. In certain embodiments, the heterologous endonuclease is engineered to have only ssDNA cleavage activity, e.g., only nickase activity, e.g., be a Cas9 nickase, e.g., SpCas9 with D10A, H840A, or N863A mutations. Table 3 provides exemplary Cas proteins and mutations associated with nickase activity. In still other embodiments, homologous endonuclease domains are modified, for example by site-specific mutation, to alter DNA endonuclease activity. In still other embodiments, endonuclease domains are modified to reduce DNA-sequence specificity, e.g., by truncation to remove domains that confer DNA-sequence specificity or mutation to inactivate regions conferring DNA-sequence specificity.

In some embodiments, the endonuclease domain has nickase activity and does not form double-stranded breaks. In some embodiments, the endonuclease domain forms single-stranded breaks at a higher frequency than double-stranded breaks, e.g., at least 90%, 95%, 96%, 97%, 98%, or 99% of the breaks are single-stranded breaks, or less than 10%, 5%, 4%, 3%, 2%, or 1% of the breaks are double-stranded breaks. In some embodiments, the endonuclease forms substantially no double-stranded breaks. In some embodiments, the endonuclease does not form detectable levels of double-stranded breaks.

In some embodiments, the endonuclease domain has nickase activity that nicks the target site DNA of the first strand; e.g., in some embodiments, the endonuclease domain cuts the genomic DNA of the target site near to the site of alteration on the strand that will be extended by the writing domain. In some embodiments, the endonuclease domain has nickase activity that

nicks the target site DNA of the first strand and does not nick the target site DNA of the second strand. For example, when a polypeptide comprises a CRISPR-associated endonuclease domain having nickase activity, in some embodiments, said CRISPR-associated endonuclease domain nicks the target site DNA strand containing the PAM site (e.g., and does not nick the target site DNA strand that does not contain the PAM site). As a further example, when a polypeptide comprises a CRISPR-associated endonuclease domain having nickase activity, in some embodiments, said CRISPR-associated endonuclease domain nicks the target site DNA strand not containing the PAM site (e.g., and does not nick the target site DNA strand that contains the PAM site).

In some other embodiments, the endonuclease domain has nickase activity that nicks the target site DNA of the first strand and the second strand. Without wishing to be bound by theory, after a writing domain (e.g., RT domain) of a polypeptide described herein polymerizes (e.g., reverse transcribes) from the heterologous object sequence of a template nucleic acid (e.g., template RNA), the cellular DNA repair machinery must repair the nick on the first DNA strand. The target site DNA now contains two different sequences for the first DNA strand: one corresponding to the original genomic DNA (e.g., having a free 5' end) and a second corresponding to that polymerized from the heterologous object sequence (e.g., having a free 3' end). It is thought that the two different sequences equilibrate with one another, first one hybridizing the second strand, then the other, and which sequence the cellular DNA repair apparatus incorporates into its repaired target site may be a stochastic process. Without wishing to be bound by theory, it is thought that introducing an additional nick to the second-strand may bias the cellular DNA repair machinery to adopt the heterologous object sequence-based sequence more frequently than the original genomic sequence (Anzalone et al. Nature 576:149-157 (2019)). In some embodiments, the additional nick is positioned at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 nucleotides 5' or 3' of the target site modification (e.g., the insertion, deletion, or substitution) or to the nick on the first strand.

Alternatively, or additionally, without wishing to be bound by theory, it is thought that an additional nick to the second strand may promote second-strand synthesis. In some embodiments, where the gene modifying system has inserted or substituted a portion of the first

strand, synthesis of a new sequence corresponding to the insertion/substitution in the second strand is necessary.

In some embodiments, the polypeptide comprises a single domain having endonuclease activity (e.g., a single endonuclease domain) and said domain nicks both the first strand and the second strand. For example, in such an embodiment the endonuclease domain may be a CRISPR-associated endonuclease domain, and the template nucleic acid (e.g., template RNA) comprises a gRNA spacer that directs nicking of the first strand and an additional gRNA spacer that directs nicking of the second strand. In some embodiments, the polypeptide comprises a plurality of domains having endonuclease activity, and a first endonuclease domain nicks the first strand and a second endonuclease domain nicks the second strand (optionally, the first endonuclease domain does not (e.g., cannot) nick the second strand and the second endonuclease domain does not (e.g., cannot) nick the first strand).

In some embodiments, the endonuclease domain is capable of nicking a first strand and a second strand. In some embodiments, the first and second strand nicks occur at the same position in the target site but on opposite strands. In some embodiments, the second strand nick occurs in a staggered location, e.g., upstream or downstream, from the first nick. In some embodiments, the endonuclease domain generates a target site deletion if the second strand nick is upstream of the first strand nick. In some embodiments, the endonuclease domain generates a target site duplication if the second strand nick is downstream of the first strand nick. In some embodiments, the endonuclease domain generates no duplication and/or deletion if the first and second strand nicks occur in the same position of the target site. In some embodiments, the endonuclease domain has altered activity depending on protein conformation or RNA-binding status, e.g., which promotes the nicking of the first or second strand (e.g., as described in Christensen et al. PNAS 2006; incorporated by reference herein in its entirety).

In some embodiments, the endonuclease domain comprises a meganuclease, or a functional fragment thereof. In some embodiments, the endonuclease domain comprises a homing endonuclease, or a functional fragment thereof. In some embodiments, the endonuclease domain comprises a meganuclease from the LAGLIDADG, GIY-YIG, HNH, His-Cys Box, or PD-(D/E) XK families, or a functional fragment or variant thereof, e.g., which possess conserved amino acid motifs, e.g., as indicated in the family names. In some embodiments, the endonuclease domain comprises a meganuclease, or fragment thereof, chosen from, e.g., I-

SmaMI (Uniprot F7WD42), I-SceI (Uniprot P03882), I-AniI (Uniprot P03880), I-DmoI (Uniprot P21505), I-CreI (Uniprot P05725), I-TevI (Uniprot P13299), I-OnuI (Uniprot Q4VWW5), or I-BmoI (Uniprot Q9ANR6). In some embodiments, the meganuclease is naturally monomeric, e.g., I-SceI, I-TevI, or dimeric, e.g., I-CreI, in its functional form. For example, the

5 LAGLIDADG meganucleases with a single copy of the LAGLIDADG motif generally form homodimers, whereas members with two copies of the LAGLIDADG motif are generally found as monomers. In some embodiments, a meganuclease that normally forms as a dimer is expressed as a fusion, e.g., the two subunits are expressed as a single ORF and, optionally, connected by a linker, e.g., an I-CreI dimer fusion (Rodriguez-Fornes et al. Gene Therapy 2020;

10 incorporated by reference herein in its entirety). In some embodiments, a meganuclease, or a functional fragment thereof, is altered to favor nickase activity for one strand of a double-stranded DNA molecule, e.g., I-SceI (K122I and/or K223I) (Niu et al. J Mol Biol 2008), I-AniI (K227M) (McConnell Smith et al. PNAS 2009), I-DmoI (Q42A and/or K120M) (Molina et al. J Biol Chem 2015). In some embodiments, a meganuclease or functional fragment thereof

15 possessing this preference for single-strand cleavage is used as an endonuclease domain, e.g., with nickase activity. In some embodiments, an endonuclease domain comprises a meganuclease, or a functional fragment thereof, which naturally targets or is engineered to target a safe harbor site, e.g., an I-CreI targeting SH6 site (Rodriguez-Fornes et al., *supra*). In some

20 embodiments, an endonuclease domain comprises a meganuclease, or a functional fragment thereof, with a sequence tolerant catalytic domain, e.g., I-TevI recognizing the minimal motif CNNNG (Kleinstiver et al. PNAS 2012). In some embodiments, a target sequence tolerant catalytic domain is fused to a DNA binding domain, e.g., to direct activity, e.g., by fusing I-TevI to: (i) zinc fingers to create Tev-ZFEs (Kleinstiver et al. PNAS 2012), (ii) other meganucleases to create MegaTevs (Wolfs et al. Nucleic Acids Res 2014), and/or (iii) Cas9 to create TevCas9

25 (Wolfs et al. PNAS 2016).

In some embodiments, the endonuclease domain comprises a restriction enzyme, e.g., a Type IIS or Type IIP restriction enzyme. In some embodiments, the endonuclease domain comprises a Type IIS restriction enzyme, e.g., FokI, or a fragment or variant thereof. In some

30 embodiments, the endonuclease domain comprises a Type IIP restriction enzyme, e.g., PvuII, or a fragment or variant thereof. In some embodiments, a dimeric restriction enzyme is expressed

as a fusion such that it functions as a single chain, e.g., a FokI dimer fusion (Minczuk et al. Nucleic Acids Res 36(12):3926-3938 (2008)).

The use of additional endonuclease domains is described, for example, in Guha and Edgell Int J Mol Sci 18(22):2565 (2017), which is incorporated herein by reference in its entirety.

In some embodiments, a gene modifying polypeptide comprises a modification to an endonuclease domain, e.g., relative to a wild-type Cas protein. In some embodiments, the endonuclease domain comprises an addition, deletion, replacement, or modification to the amino acid sequence of the wild-type Cas protein. In some embodiments, the endonuclease domain is modified to include a heterologous functional domain that binds specifically to and/or induces endonuclease cleavage of a target nucleic acid (e.g., DNA) sequence of interest. In some embodiments, the endonuclease domain comprises a zinc finger. In embodiments, the endonuclease domain comprising the Cas domain is associated with a guide RNA (gRNA), e.g., as described herein. In some embodiments, the endonuclease domain is modified to include a functional domain that does not target a specific target nucleic acid (e.g., DNA) sequence. In embodiments, the endonuclease domain comprises a FokI domain.

In some embodiments, the endonuclease domain is associated with the target dsDNA *in vitro* at a frequency at least about 5-fold or 10-fold higher than with a scrambled dsDNA. In some embodiments, the endonuclease domain is associated with the target dsDNA *in vitro* at a frequency at least about 5-fold or 10-fold higher than with a scrambled dsDNA, e.g., in a cell (e.g., a HEK293T cell). In some embodiments, the frequency of association between the endonuclease domain and the target DNA or scrambled DNA is measured by ChIP-seq, e.g., as described in He and Pu (2010) *Curr. Protoc Mol Biol* Chapter 21 (incorporated by reference herein in its entirety).

In some embodiments, the endonuclease domain can catalyze the formation of a nick at a target sequence, e.g., to an increase of at least about 5-fold or 10-fold relative to a non-target sequence (e.g., relative to any other genomic sequence in the genome of the target cell). In some embodiments, the level of nick formation is determined using NickSeq, e.g., as described in Elacqua et al. (2019) *bioRxiv* doi.org/10.1101/867937 (incorporated herein by reference in its entirety).

In some embodiments, the endonuclease domain is capable of nicking DNA *in vitro*. In embodiments, the nick results in an exposed base. In embodiments, the exposed base can be detected using a nuclease sensitivity assay, e.g., as described in Chaudhry and Weinfeld (1995) *Nucleic Acids Res* 23(19):3805-3809 (incorporated by reference herein in its entirety). In
 5 embodiments, the level of exposed bases (e.g., detected by the nuclease sensitivity assay) is increased by at least 10%, 50%, or more relative to a reference endonuclease domain. In some embodiments, the reference endonuclease domain is an endonuclease domain from Cas9 of *S. pyogenes*.

In some embodiments, the endonuclease domain is capable of nicking DNA in a cell. In
 10 embodiments, the endonuclease domain is capable of nicking DNA in a HEK293T cell. In embodiments, an unrepaired nick that undergoes replication in the absence of Rad51 results in increased NHEJ rates at the site of the nick, which can be detected, e.g., by using a Rad51 inhibition assay, e.g., as described in Bothmer et al. (2017) *Nat Commun* 8:13905 (incorporated by reference herein in its entirety). In embodiments, NHEJ rates are increased above 0-5%. In
 15 embodiments, NHEJ rates are increased to 20-70% (e.g., between 30%-60% or 40-50%), e.g., upon Rad51 inhibition.

In some embodiments, the endonuclease domain releases the target after cleavage. In some embodiments, release of the target is indicated indirectly by assessing for multiple turnovers by the enzyme, e.g., as described in Yourik et al. *RNA* 25(1):35-44 (2019)
 20 (incorporated herein by reference in its entirety) and shown in FIG. 2. In some embodiments, the k_{exp} of an endonuclease domain is $1 \times 10^{-3} - 1 \times 10^{-5} \text{ min}^{-1}$ as measured by such methods.

In some embodiments, the endonuclease domain has a catalytic efficiency (k_{cat}/K_m) greater than about $1 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$ *in vitro*. In embodiments, the endonuclease domain has a catalytic efficiency greater than about 1×10^5 , 1×10^6 , 1×10^7 , or 1×10^8 , $\text{s}^{-1} \text{ M}^{-1}$ *in vitro*. In
 25 embodiments, catalytic efficiency is determined as described in Chen et al. (2018) *Science* 360(6387):436-439 (incorporated herein by reference in its entirety). In some embodiments, the endonuclease domain has a catalytic efficiency (k_{cat}/K_m) greater than about $1 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$ in cells. In embodiments, the endonuclease domain has a catalytic efficiency greater than about 1×10^5 , 1×10^6 , 1×10^7 , or $1 \times 10^8 \text{ s}^{-1} \text{ M}^{-1}$ in cells.

Gene modifying polypeptides comprising Cas domains

In some embodiments, a gene modifying polypeptide described herein comprises a Cas domain. In some embodiments, the Cas domain can direct the gene modifying polypeptide to a target site specified by a gRNA spacer, thereby modifying a target nucleic acid sequence in

5 “cis.” In some embodiments, a gene modifying polypeptide is fused to a Cas domain. In some embodiments, a gene modifying polypeptide comprises a CRISPR/Cas domain (also referred to herein as a CRISPR-associated protein). In some embodiments, a CRISPR/Cas domain comprises a protein involved in the clustered regulatory interspaced short palindromic repeat (CRISPR) system, e.g., a Cas protein, and optionally binds a guide RNA, e.g., single guide RNA

10 (sgRNA).

CRISPR systems are adaptive defense systems originally discovered in bacteria and archaea. CRISPR systems use RNA-guided nucleases termed CRISPR-associated or “Cas” endonucleases (e. g., Cas9 or Cpf1) to cleave foreign DNA. For example, in a typical CRISPR-Cas system, an endonuclease is directed to a target nucleotide sequence (e. g., a site in the

15 genome that is to be sequence-edited) by sequence-specific, non-coding “guide RNAs” that target single- or double-stranded DNA sequences. Three classes (I-III) of CRISPR systems have been identified. The class II CRISPR systems use a single Cas endonuclease (rather than multiple Cas proteins). One class II CRISPR system includes a type II Cas endonuclease such as Cas9, a CRISPR RNA (“crRNA”), and a trans-activating crRNA (“tracrRNA”). The crRNA

20 contains a “spacer” sequence, a typically about 20-nucleotide RNA sequence that corresponds to a target DNA sequence (“protospacer”). In the wild-type system, and in some engineered systems, crRNA also contains a region that binds to the tracrRNA to form a partially double-stranded structure that is cleaved by RNase III, resulting in a crRNA/tracrRNA hybrid molecule. A crRNA/tracrRNA hybrid then directs the Cas endonuclease to recognize and cleave a target

25 DNA sequence. A target DNA sequence is generally adjacent to a “protospacer adjacent motif” (“PAM”) that is specific for a given Cas endonuclease and required for cleavage activity at a target site matching the spacer of the crRNA. CRISPR endonucleases identified from various prokaryotic species have unique PAM sequence requirements, e.g., as listed for exemplary Cas enzymes in Table 3; examples of PAM sequences include 5′-NGG (*Streptococcus pyogenes*), 5′-

30 NNAGAA (*Streptococcus thermophilus* CRISPR1), 5′-NGGNG (*Streptococcus thermophilus* CRISPR3), and 5′-NNNGATT (*Neisseria meningitidis*). Some endonucleases, e.g., Cas9

endonucleases, are associated with G-rich PAM sites, e. g., 5'-NGG, and perform blunt-end cleaving of the target DNA at a location 3 nucleotides upstream from (5' from) the PAM site. Another class II CRISPR system includes the type V endonuclease Cpf1, which is smaller than Cas9; examples include AsCpf1 (from *Acidaminococcus* sp.) and LbCpf1 (from *Lachnospiraceae* sp.). Cpf1-associated CRISPR arrays are processed into mature crRNAs without the requirement of a tracrRNA; in other words, a Cpf1 system, in some embodiments, comprises only Cpf1 nuclease and a crRNA to cleave a target DNA sequence. Cpf1 endonucleases, are typically associated with T-rich PAM sites, e. g., 5'-TTN. Cpf1 can also recognize a 5'-CTA PAM motif. Cpf1 typically cleaves a target DNA by introducing an offset or staggered double-strand break with a 4- or 5-nucleotide 5' overhang, for example, cleaving a target DNA with a 5-nucleotide offset or staggered cut located 18 nucleotides downstream from (3' from) from a PAM site on the coding strand and 23 nucleotides downstream from the PAM site on the complimentary strand; the 5-nucleotide overhang that results from such offset cleavage allows more precise genome editing by DNA insertion by homologous recombination than by insertion at blunt-end cleaved DNA. See, e.g., Zetsche et al. (2015) *Cell*, 163:759 – 771.

A variety of CRISPR associated (Cas) genes or proteins can be used in the technologies provided by the present disclosure and the choice of Cas protein will depend upon the particular conditions of the method. Specific examples of Cas proteins include class II systems including Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9, Cas10, Cpf1, C2C1, or C2C3. In some embodiments, a Cas protein, e.g., a Cas9 protein, may be from any of a variety of prokaryotic species. In some embodiments a particular Cas protein, e.g., a particular Cas9 protein, is selected to recognize a particular protospacer-adjacent motif (PAM) sequence. In some embodiments, a DNA-binding domain or endonuclease domain includes a sequence targeting polypeptide, such as a Cas protein, e.g., Cas9. In certain embodiments a Cas protein, e.g., a Cas9 protein, may be obtained from a bacteria or archaea or synthesized using known methods. In certain embodiments, a Cas protein may be from a gram-positive bacteria or a gram-negative bacteria. In certain embodiments, a Cas protein may be from a *Streptococcus* (e.g., a *S. pyogenes*, or a *S. thermophilus*), a *Francisella* (e.g., an *F. novicida*), a *Staphylococcus* (e.g., an *S. aureus*), an *Acidaminococcus* (e.g., an *Acidaminococcus* sp. BV3L6), a *Neisseria* (e.g., an *N. meningitidis*), a *Cryptococcus*, a *Corynebacterium*, a *Haemophilus*, a *Eubacterium*, a *Pasteurella*, a *Prevotella*, a *Veillonella*, or a *Marinobacter*.

In some embodiments, a gene modifying polypeptide may comprise a Cas domain as listed in Table 3 or 4, or a functional fragment thereof, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity thereto.

5 **Table 3. CRISPR/Cas Proteins, Species, and Mutations**

Name	Enzyme	Species	# of AAs	PAM	Mutations to alter PAM recognition	Mutations to make catalytically dead
<i>FnCas9</i>	Cas9	<i>Francisella novicida</i>	1629	5'-NGG-3'	Wt	D11A/H969A/N995A
<i>FnCas9 RHA</i>	Cas9	<i>Francisella novicida</i>	1629	5'-YG-3'	E1369R/E1449H/R1556A	D11A/H969A/N995A
<i>SaCas9</i>	Cas9	<i>Staphylococcus aureus</i>	1053	5'-NNGRRT-3'	Wt	D10A/H557A
<i>SaCas9 KKH</i>	Cas9	<i>Staphylococcus aureus</i>	1053	5'-NNNRRT-3'	E782K/N968K/R1015H	D10A/H557A
<i>SpCas9</i>	Cas9	<i>Streptococcus pyogenes</i>	1368	5'-NGG-3'	Wt	D10A/D839A/H840A/N863A
<i>SpCas9 VQR</i>	Cas9	<i>Streptococcus pyogenes</i>	1368	5'-NGA-3'	D1135V/R1335Q/T1337R	D10A/D839A/H840A/N863A
<i>AsCpf1 RR</i>	Cpf1	<i>Acidaminococcus</i> sp. BV3L6	1307	5'-TYCV-3'	S542R/K607R	E993A
<i>AsCpf1 RVR</i>	Cpf1	<i>Acidaminococcus</i> sp. BV3L6	1307	5'-TATV-3'	S542R/K548V/N552R	E993A
<i>FnCpf1</i>	Cpf1	<i>Francisella novicida</i>	1300	5'-NTTN-3'	Wt	D917A/E1006A/D1255A
<i>NmCas9</i>	Cas9	<i>Neisseria meningitidis</i>	1082	5'-NNNGATT-3'	Wt	D16A/D587A/H588A/N611A

Table 4 Amino Acid Sequences of CRISPR/Cas Proteins, Species, and Mutations

Variant	Parental Host(s)	Protein Sequence	Nickase (HNH)	Nickase (HNH)	Nickase (RuvC)
Nme2Cas9	<i>Neisseria meningitidis</i>	MAAFKPNPYNILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFVFERAEVPK TGDSLAMARRLARSVRRLTRRRHRLRLARRLLKREGVLQAADFENGLIKS LPNTPWQLRAAALDRKLTLEWSAVLLHLIKHRGYLSQRKNEGETADKELG ALLKGVANNAHALQTGDFRTPAELALNKFESGHIRNQRGDYSHTFSRKD LQAEILLFEKQKEFGNPHVSGGLKEGIEITLLMTQRPALSGDAVQKMLGHCT FEPAPKAAKNTYTAERFIWLTNNLRILEQGSERPLDTERATLMDEPYRK SKLTQAQARKLLGLEDTAFFKGLRYGKDNEASTLMEMKAYHAISRALEKEG LKDKKSPLNLSSELQDEIGTAFSLFKTDEITGRKDRVQPEILEALLKHISFDKF VQISLKALRRIVPLMEQGGKRYDEACAIEYGDHYGKNTKEIYLPPIPADEIRN PVVLRALSQARKVINGVVRYPARIHETAREVGSKFDRKEIEKRQEENR KDREKAAAKFREYFPNFVGEPSKDIKLRLYEQQHGKCLYSKGKINLVRLNE KGYVEIDHALPFSRTWDDSFNNKVLVLSSENQNGNQTPEYFNGKDNRSR EWQEFKARVETSRFPRSKQRILLQKFDGFKECNLNDRYVNRFLCQFVA DHILLTGKGRVVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVACS TVAMQQKITRFVRYKEMNAFDGKTIDKTKGVLHQTHTFPQPWEFFAQEV MIRVFGKPDGKPEFEADTPEKRLTLAEKLSSRPEAVHEYVTLFVSRAPNR KMSGAHKDTLSAKRFVKNHNEISVKRVWLTEIKLADLENMVMYKNGREIEL YEALKARLEAYGGNAKQAFDPKDNPFYKGGQLKAVRVEKTQESGVLLNK KNAYTIADNGDMVRVDVFCVKDKGKNQYFIVPIYAWQVAENILPDIDCKG YRIDDSYTFCSLHKYDLIAFQKDEKSKVEFAYINCDSSNGRFLAWHDKGS KEQQFRISTQNLVLIQYQVNLGKEIRPCRLKKRPPVR	N611A	H588A	D16A
PpnCas9	<i>Pasteurella pneumotropica</i>	MQNNPLNLYLGLDIGIASIGWAVVEIDEESSPIRLIDVGVRTFERAEVAKTGE SLALSRRLARSSRLIKRAERLKKAKRLLKAEILHSIDEKLPINWQLRVKGL KEKLERQEWAAVLLHLSKHRGYLSQRKNEGKSDNKELGALLSGIASNHQML QSSEYRTPAEIAVKKFQVEEGHIRNQGSYTHFSRLDLAEMELLFQRQAEL	N605A	H582A	D13A

		GNSYTS TTLLENLTALLMWQK PALAGDAILKMLGKCTFEPSEYKAAKNSYSA ERFVWLTKLNNLRILENGTERALNDNERFALLEQPYEKSCLTYAQVRAMLAL SDNAIFKGVRYLGEDKKTVESKTTLIEMKFYHQIRKTLGSALKEWNEKGN SDLLDEIGTAFSLYKTD DDICRYLEGKLPERVLNALLNLFDFIQLSLKALHQ ILPLMLQGQRYDEAVSAIYGDHYGKKSTETTRLLPTIPADEIRNPVVLRTLTQA RKVINAVVRLYGSPARIHIETAREVGSYQDRKKLEKQEDNRKQRESAVKK FKEMFPHFVGEKPKGDILKMRLEYLQQAACLYSGKSLELHRLLEKGYVEVDH ALPFSRTWDDSFNNKVLVLANENQNKGNLTPYEWLDGKNNSERWQHFFVV RVQTSGFSYAKKQRIINHKLDEKGFIERNLNDTRYVARFLCNFIADNMLLVG KGRNVFASNGQITALLRHRWGLQKVREQNDRHHALDAVVACSTVAMQ QKITRFVRYNEG NVFSGERIDRETGEIPLHFPSPWAFFKENVEIRFSENPKLE LENRLPDYPQYNHEWVQPLFVSRMPTRKMTQGGMETVKSARLNEGLS VLKVP LTLQKLSDLERMVNRDREIALYESLKARLEQFGNDPAKAFAPFYKKG GALVKAVRLEQTQKSGVLVRDGNGVADNASMVRVDVFTGGKYFLVPIYT WQVAKGILPNRAATQGDENDWDIMDEMATFQFSLCQNDLIKLVTKKKT FGYFNGLN RATSNINIKEHDLDSKSGKLG IYLEVGVKL AISLEKYQVDELGKNI RPCRPTKRQHVR			
SauCas9	Staphylococcus aureus	MKRNYILGLDIGITSVG YGIIDYETRDVIDAGVRLFEANVENNEGRRSKRGAR RLKRRRRHRIQRVKLLFDYNLLTDHSELGINPYEARVKGLSQKLEEEFSA ALLHLAKRRGVHNVNEVEEDTGNELSTKEQJSRNSKALEEKYVAELQLERLKK DGEVRSINRFKTS DYVKEAKQLLVQKAYHQLDQSFDITYIDLETRRTYYE GPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAE LLDQIAKILTIYQSSEDIQEELTNLNSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPKKV DLSQQKEIPTTLVD FILSPVVKRSFIQSKVINAIKKYGLPNDIIELAREKNSK DAQKMINEMQKRNQRTNERIEIIRTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIIPRSVSFDNSFNKVLVKQEENSKKGNRTPFQ YLSSSDSKISYETFKKHILNLAGKGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHAEDALI ANADFIKWKLDKAKKVMENQMFEKQAE SMPEIETEQ EYKEIFITPHQIKHKDFKDYKSHRVDKKPNRELINDTLYSTRKDDKGN TLIVN NNLGLYDKDNDKLLINKSPEKLLMYHHPQTYQKLKLIMEQYGDENPL YKYEEETGNLYTKYSKKNPVIKKIKYGNKLAHL DITDDYPNSRNKVVKL SLKPYRFDVYLDNGVYKFVTVKNLDVIKENYEVNSKCYEEAKLKKISNQA EFIASFYNNDLIKINGELYRVIGVNNDLNRIEVNMIDITYREYLENMNDKRP RIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIKKG	N580A	H557A	D10A
SauCas9-KKH	Staphylococcus aureus	MKRNYILGLDIGITSVG YGIIDYETRDVIDAGVRLFEANVENNEGRRSKRGAR RLKRRRRHRIQRVKLLFDYNLLTDHSELGINPYEARVKGLSQKLEEEFSA ALLHLAKRRGVHNVNEVEEDTGNELSTKEQJSRNSKALEEKYVAELQLERLKK DGEVRSINRFKTS DYVKEAKQLLVQKAYHQLDQSFDITYIDLETRRTYYE GPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAE LLDQIAKILTIYQSSEDIQEELTNLNSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPKKV DLSQQKEIPTTLVD FILSPVVKRSFIQSKVINAIKKYGLPNDIIELAREKNSK DAQKMINEMQKRNQRTNERIEIIRTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIIPRSVSFDNSFNKVLVKQEENSKKGNRTPFQ YLSSSDSKISYETFKKHILNLAGKGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHAEDALI ANADFIKWKLDKAKKVMENQMFEKQAE SMPEIETEQ EYKEIFITPHQIKHKDFKDYKSHRVDKKPNRKLINDTLYSTRKDDKGN TLIV NNLGLYDKDNDKLLINKSPEKLLMYHHPQTYQKLKLIMEQYGDENPL LYKYEEETGNLYTKYSKKNPVIKKIKYGNKLAHL DITDDYPNSRNKVVK LSKPYRFDVYLDNGVYKFVTVKNLDVIKENYEVNSKCYEEAKLKKISNQA AEFIASFYKNDLIKINGELYRVIGVNNDLNRIEVNMIDITYREYLENMNDKRP PHIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIKKG	N580A	H557A	D10A

SauriCas9	Staphylococcus auricularis	MQENQQKQNYILGLDIGITSVGYGLIDSKTREVIDAGVRLFPEADSENNNSNR RSKRGAARRLKRRIHRLNVRKDLLADYQMDLNNVPKSTDPYTIRVKGLEPL TKEEFAIALLHIAKRRGLHNISVSMGDEEQDNELSTKQLQKNAQQQLQDKY VCELQLERLTNINKVRGEKNRKFTEDFVKEVKQLCETQRQYHNDDQFIQQY IDLVSTRREYFEGPGNGSPYGDGDLKWKYKLMGRCTYFPEELRSVKYAYS ADLFNALNDLNNLVVTRDDNPKEYYEKYHIIENVFKQKKNPTLKQIAKEIGV QDYDIRGYRITKSGKPQFTSFKLYHDLKNIFEQAKYLEDVEMLEIAKILTIYQ DEISIKKALDQLPELLTESEKSQIAQLTGYTGTHRLSLKCIHIVIDELWESPENQ MEIFTRLNLKPKKVEMSEIDSIPTTLVDEFILSPVVKRAFIQSIKVINAVINRFL PEDIHILAREKNSKDRRKFINLQKQNEATRKKIEQLLAKYGNNTAKYMIEKI KLHDMQEGKCLYSLEAIPLEDLLSNPTHYEVVDHIIPRSVSFDNSLNNKVLVKQ SENSKKGNRTPYQYLSSNESKISYNQFKQHILNLSKAKDRISKKKRDMLEER DINKFEVQKEFINRNLVDTRYATRELSNLLKTYFSTHDYAVKVKTINGGFTNH LRKVWDFKKHRNHGYKHAEDALVIANADFLKTHKALRRTDKILEQPGLE VNDTTVKVDTEEKYQELFETPKQVKNIKQFRDFKYSHRVDKKPNRQLINDTL YSTREIDGETYVVTQLKDLYAKDNEKVKKLFTERPQKILMYQHDPKTFEKL TILNQYAEAKNPLAAYYEDKGEYVTKYAKKGNGPAIHKIKYIDKLGSLDVS NKYPETQNKLVKLSKSRFDIYKCEQGYKMVSIGYDLVLKKNYYIYPKDKYE AEKQKKIKESDLFVGSFYNDLIMYEDELFRVIGVNSDINNVLNLMVDITY KDFCEVNNVTGEKRIKKTIGKRVVLEKYTTDILGNLYKTPLPKKPQLIFKRGEL	N588A	H565A	D15A
SauriCas9- KKH	Staphylococcus auricularis	MQENQQKQNYILGLDIGITSVGYGLIDSKTREVIDAGVRLFPEADSENNNSNR RSKRGAARRLKRRIHRLNVRKDLLADYQMDLNNVPKSTDPYTIRVKGLEPL TKEEFAIALLHIAKRRGLHNISVSMGDEEQDNELSTKQLQKNAQQQLQDKY VCELQLERLTNINKVRGEKNRKFTEDFVKEVKQLCETQRQYHNDDQFIQQY IDLVSTRREYFEGPGNGSPYGDGDLKWKYKLMGRCTYFPEELRSVKYAYS ADLFNALNDLNNLVVTRDDNPKEYYEKYHIIENVFKQKKNPTLKQIAKEIGV QDYDIRGYRITKSGKPQFTSFKLYHDLKNIFEQAKYLEDVEMLEIAKILTIYQ DEISIKKALDQLPELLTESEKSQIAQLTGYTGTHRLSLKCIHIVIDELWESPENQ MEIFTRLNLKPKKVEMSEIDSIPTTLVDEFILSPVVKRAFIQSIKVINAVINRFL PEDIHILAREKNSKDRRKFINLQKQNEATRKKIEQLLAKYGNNTAKYMIEKI KLHDMQEGKCLYSLEAIPLEDLLSNPTHYEVVDHIIPRSVSFDNSLNNKVLVKQ SENSKKGNRTPYQYLSSNESKISYNQFKQHILNLSKAKDRISKKKRDMLEER DINKFEVQKEFINRNLVDTRYATRELSNLLKTYFSTHDYAVKVKTINGGFTNH LRKVWDFKKHRNHGYKHAEDALVIANADFLKTHKALRRTDKILEQPGLE VNDTTVKVDTEEKYQELFETPKQVKNIKQFRDFKYSHRVDKKPNRKLINDTL YSTREIDGETYVVTQLKDLYAKDNEKVKKLFTERPQKILMYQHDPKTFEKL TILNQYAEAKNPLAAYYEDKGEYVTKYAKKGNGPAIHKIKYIDKLGSLDVS NKYPETQNKLVKLSKSRFDIYKCEQGYKMVSIGYDLVLKKNYYIYPKDKYE AEKQKKIKESDLFVGSFYNDLIMYEDELFRVIGVNSDINNVLNLMVDITY KDFCEVNNVTGEKHIKKTIGKRVVLEKYTTDILGNLYKTPLPKKPQLIFKRGEL	N588A	H565A	D15A
ScaCas9- Sc++	Streptococcus canis	MEKKSIGLDIGTNSVGWAVITDDYKVPSSKFKVLGNTNRKSIKKNLMGALL FDSGETAEATRLKRTARRRYTRRKNRIRYLQEIFANEMAKLDDSFQRLSEESF LVEEDKKNERHPIFGNLADEVAYHRNYPYTIYHLRKKLADSPEKADRLIYLALA HIIKFRGHFLIEGKLNANSDVAKLFYQLIQTYNQLFEESPLDEIEVDAKGILSA RLSKSRLEKLIAVFPNEKKNLFGNIIALALGLTPNFKSNFDLTEDAKLQLSKD TYDDDLDELLGQIGDQYADLFSAAKNLSDAILLSDILRSNSEVTKAPLSASMV KRYDEHHQDLALLKTLVRQQFPEKYAEIFKDDTKNGYAGYVGADKKLRKRS GKLATEEEFYKFIKPILEKMDGAELLAKLNRDILLRKQRTFDNGSIPHQIHLK ELHAILRRQEEFYFPFLKENREKIEKILTRIPYVVGPLARGNSRFAWLTRKSEE ITPWNFEVVDKGASAQSFIERMTNFDEQLPNKKVLPKHSLLYEYFTVYNEL TKVKYVTERMRKPEFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS VEIIGVEDRFNASLGYHDLKIKKDKDFLDNEENEDILEDIVLTLTLFEDREMIE ERLKYAHLFDKVMKQLKRRHYTGWGRLSRKMINGIRDQSGKTILDFLKS DGFNRRNFMQLIHDDSLTFKEEIEKAQVSGQDLSLHEQIADLAGSPAIKKGIL QTVKIVDELVKVMGHKPENIVIAMARENQTTTGLQQSREKRIEIGIKELE SQILKENPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYVDVHIVP QSFIDDSIDNKVLTBSVENRGKSDNPSEEVVKKMKNYWRQLLNAKLITQ RKFDNLTKAERGGLEADKAGFIKRLVETRQITKHVARILDSRMNTKRDKN DKPIREVKVITLKSCLVSDFRKDFQLYKVRDINNYHHAHDAYLNAVVGTA LIK	N872A	H849A	D10A

		KYPKLESEFVYGDYKVVYDVRKMIKSEQEIGKATAKRRFFYSNIMNFFKTEVKL ANGEIRKRPLIETNGETGEVWVNKEKDFATVRKVLAMPQVNIIVKTEVQTG GFSKESILSKRESAKLIPRKGWDTRKYGGFGSPTVAYSILVVAKEKGAKKL KSVKVLVGITIMEKGSYEKDPGFLEAKGYKDIKKELIFKLPKYSLEENGRRR MLASAKELQKANELVLPQHLVRLYYTQNISATTGSNNLGYIEQHREEFKEIF EKIIDFSEKYLKKNVNSNLKSSFDEQFAVSDSILLSNSFVSLKYTSFGASGGFT FLDLVDKQGRRLRYQTVTEVLDATLIYQISITGLYETRTDLSQLGGD			
SpyCas9	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRNRCYLQEIFSNEMAKVDDSFHRLSEESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRILIYALAH MIKFRGHFLIEGDLNPDNSVDVDFIQLVQTYNQLFEENPINASGVDAKAILS ARLSKSRRENLIQALPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSAAILSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKTYA HLFDDKVMKQLKRRRYTGWRSLRKLINGIRDKQSGKTILDFLKSDFGANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIAKKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEIGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNKVLRSDKNRGKSDNVPSEEVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSELDAKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKYPK LESEFVYGDYKVVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEI RKRPLIETNGETGEVWVNKEKDFATVRKVLAMPQVNIIVKTEVQTGFGFSKES ILPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSILVVAKEKGKSKKLSVKE LLGITIMERSSEKKNPIDFLEAKGYKEVKDLIILPKYSLEENGRRKRLASA GELQKGNELALPSKYVNFYLAHYEKLKGPEDNEQKQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHFTLTNLGAPAAF KYFDTTIDRKRYTSTKEVL DATLIHQSI TGLYETRIDLSQLGGD	N863A	H840A	D10A
SpyCas9- NG	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRNRCYLQEIFSNEMAKVDDSFHRLSEESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRILIYALAH MIKFRGHFLIEGDLNPDNSVDVDFIQLVQTYNQLFEENPINASGVDAKAILS ARLSKSRRENLIQALPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSAAILSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKTYA HLFDDKVMKQLKRRRYTGWRSLRKLINGIRDKQSGKTILDFLKSDFGANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIAKKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEIGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNKVLRSDKNRGKSDNVPSEEVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSELDAKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKYPK LESEFVYGDYKVVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEI RKRPLIETNGETGEVWVNKEKDFATVRKVLAMPQVNIIVKTEVQTGFGFSKES IRPKRNSDKLIARKKDWDPKKYGGFVSPTVAYSILVVAKEKGKSKKLSVKE LLGITIMERSSEKKNPIDFLEAKGYKEVKDLIILPKYSLEENGRRKRLASA RFLQKGNELALPSKYVNFYLAHYEKLKGPEDNEQKQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHFTLTNLGAPRAF KYFDTTIDRKVYRSTKEVL DATLIHQSI TGLYETRIDLSQLGGD	N863A	H840A	D10A

SpyCas9-SpRY	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAERTRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFL VEEDKKHERHPFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAH MIKFRGHFLIEGDLNPDNSDVDFLFIQLVQTYNQLFEENPINASGVDAKAILS ARLSKSRRLENLIAQLPGEKKNGLFGNLJALSGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EYVDKGASQAQSIERMNTNFDKNLPNEKVLPHKSHLLYEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEEDILEDIVLTLTLFEDREMIEERLKYTA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDQKSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIKKGILQTVKV VDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDHDHIVPQSF LKDDSIDNKVLTNRSDKNRGSNDVPSEVVKMKMKNYWRQLLNAKLITQRKF DNLTKAERGGSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSLSVDFRQDFQFYKVRINNYYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEIKAATKATKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVSMPQVNVKKEVQTTGGFSKES IRPKRNSDKLIARKKDWDPKKYGGFLWPTVAYSVLVAKVEKGSKKLKSVK ELLGITIMERSSEKPNIDFLEAKGYKEVKDLIILPKYSLEENGRKRMLAS AKQLQKGNELALPSKYVNFYLAHYEKLKSGSPEDNEQKQLFVEQHKHYLDE IIEQISEFSKRVILADANLDKVSAYNKHDKPIREQAENIIHLFTLTRLGAPRAF KYFDTTIDPKQYRSTKEVLDTLIHQISITGLYETRIDLSQLGGD	N863A	H840A	D10A
St1Cas9	Streptococcus thermophilus	MSDLVLGLDIGISVGVGILNKVTGEIHKNSRIFPAAQAENNLVRRTNRQG RRLARRKKHRRVRLNRLFEEESGLITDFTKISINLNPYQLRVKGLTDELSNEELFI ALKNMVVKHRGISYLDASDDGNSSVGDYQIVKENSQLETKTPGQIQLER YQTYGQLRGDFTVEKDGKKHRLINVFPTSAYRSEALRILQTQQFNPQITDEF INRYLEILTGRKRYHGPNGEKSRTDYGRYRTSGETLDNIFGILIGKCTFYPPDEF RAAKASYTAQEFNLLNDLNNLTVPTETKLSKEQKNQIINYVKNEKAMGPAK LFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKLTLETLDIEQMDRET DKLAYVLTNTEREGIQEALHEFADGSFSQKQVDELVQFRKANSSIFGKGW HNFVSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEEIY NPVVAKSVRQAIVNAAIKYGFDFNIVIEMARETNEDDEKKAQIKQKAN KDEKDAAMLKAANQYNGKAEPLHSVFHGHKLATKIRLWHQQGERCLYT GKTISIHDLINNSNQFEVDHILPLSITFDDSLANKVLVYATANQEKGGQRTPYQ ALDSMDDAWSFRELKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNLV DTRYASRVVLNALQEHFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDTYH HHAVDALIAASSQLNLWKKQKNTLVSYSEDQLLDIETGELISDDEYKESVFK APYQHFDVTLKSKEFEDSILFSYQVDSKFNKISDATIYATRQAKVGGDKADE TYVLGKIKDIYTDGYDAFMKIYKDKSKFLMYRHDPTFEKVIEPILENYPN KQINEKGKEVPCNPFLKYKEEHGYIRKYSKKGNGPEIKSLKYDCKLGNHIDIT PKDSNNKVVQLQSVSPWRADVYFNKTTGKYELGLKYADLQFEKGTGTYKISQ EKYNDIKKKEGVDSSEFKFTLYKNDLLLVKDTETKEQQLFRFLSRTMPKQKH YVELKPYDKQKFEGGEALIKVLGNVANSQGCKKGLGKSNISIYKVRTDVLGN QHIIKNEGDKPKLDF	N622A	H599A	D9A
BlatCas9	Brevibacillus laterosporus	MAYTMGIDVGIASCGWAIVDLERQRIIDIGVRTFEKAENPKNGEALAVPRRE ARSSRRRLRRKKHRIERLKHMFVRNGLAVDIQHLEQTLRSQNEIDVWQLRV DGLDRMLTQKEWLRVLIHLAQRGFGQSNRKTGSGSEDGQVLVNVNTENDRL MEEKDYRTVAEMMMVKDEKFSDHKRKNNGNYHGVVSRSSLVEIHTLFETQ RQHHSNLSKDFELEYVNIWSAQRPVATKDQIEKMIGTCTFLPKKRAPKAS WHFQYFMLLQTINHIRITNVQGTSLNKEEIEQVVMALTKSVSYHDTRKI LDLSEYQFVGLDYGKEDEKKVESKETIILDDYHKLNIKIFNEVELAKGETWE ADDYDTVAYALTFFKDDEJRDYLNKYKDSKNRLVKNLANKKEYTNELIGKV STLSFRKVGHLSLALRKIIPFLEQGMTYDKACQAAAGDFQGISKKRSVVL VIDQISNPVVNRALTQTRKVINALKYKGPETIHIETARELSKTFDERKNITKD YKENRDKNEHAKHLSLGIINPTGLDIVKYLWCEQQGRCMYSNQPISEFER	N607A	H584A	D8A

		LKESGYTEVDHIIPYSRSMNDSYNNRVLMTRENREKGNQTPFEYMGNDT QRWYEFQQRVTTNPQJKEKQNLKLGFTNRREMLELRNLNDTRYITKYL SHFISTNLEFSPDKKKVVNTSGRITSHLSRWGLEKNRGQNDLHHAMDAI VIAVTSDFSQVQVNTYKRKRELNGDDKFLPWKFFREEVIARLSPNPKEQ IEALPNHFYSEDELADLPFVSRMPKRSITGEAHQAQFRRVVGKTKEGKNIT AKKTALVDISYDKNGDFNMYGRETDPATYEAIKERYLEFGGNVKKAFSTDLH KPKKDGTKGPKLSVRIMENKTLVHPVNGKGGVYNSIVRTDVFQRKEYY LLPVYVTDVTKGKLPNKVIVAKKGYHDWIEVDDSTFLFSLYPNDLIFIRQNP KKISLKKRIESHSISDSKEVQEIHAYYKGVDSSTAIEFIHDGSYYAKGVGVQN LDCFEKYQVDILGNYFKVKGKRELETSDSNHKGKDVNSIKSTS			
cCas9-v16	Staphylococcus aureus	MKRNYILGLDIGITSVGYGIIYETRDVIDAGVRLFEANVENNEGRRSKGA RRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGSLQKLEEEFSA ALLHLAKRRGVHNVNEEEDTGNELSTKEQISRNKALEEKYVAELQLERLKK DGEVRGSINRFKTSDYVKEAKQLLKVKAYHQLDQSFIDTYIDLLETRRTYYE GPGEPSFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTYQSSEDIQEELTNLSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPPKV DLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSK DAQKMINEMQKRNQRTNERIEIIRTGTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIPRSVSFDNSFNKVLVKQENSKKGNRTPFQ YLSSDSKISYETFKKHILNLAGKGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHHAEDALIADNADFIKEWKKLDKAKKVMENQMFEKQAESMPETEIQ EYKEIFITPHQKHIDFKDYKSHRVDKPNRKLINDTLYSTRKDDKGNTLIV NNLNGLYDKDNDKLLKLINKSPEKLLMYHDPQTYQKLKLIMEQYGDENP LYKYYEETGNLYTKYSKDNQGVKIKYKYNKLNALDITDDYPNSRNKVVK LSLKPYPYFDVYLDNGVYKFTVKNLDVIKKENYEVNSKCYEAKKLLKISNQ AEFIASFYKNDLIKINGELYRVIGVNSDKNNLIEVNMIDITYREYLENMNDKRP PHIHTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG	N580A	H557A	D10A
cCas9-v17	Staphylococcus aureus	MKRNYILGLDIGITSVGYGIIYETRDVIDAGVRLFEANVENNEGRRSKGA RRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGSLQKLEEEFSA ALLHLAKRRGVHNVNEEEDTGNELSTKEQISRNKALEEKYVAELQLERLKK DGEVRGSINRFKTSDYVKEAKQLLKVKAYHQLDQSFIDTYIDLLETRRTYYE GPGEPSFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTYQSSEDIQEELTNLSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPPKV DLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSK DAQKMINEMQKRNQRTNERIEIIRTGTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIPRSVSFDNSFNKVLVKQENSKKGNRTPFQ YLSSDSKISYETFKKHILNLAGKGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHHAEDALIADNADFIKEWKKLDKAKKVMENQMFEKQAESMPETEIQ EYKEIFITPHQKHIDFKDYKSHRVDKPNRKLINDTLYSTRKDDKGNTLIV NNLNGLYDKDNDKLLKLINKSPEKLLMYHDPQTYQKLKLIMEQYGDENP LYKYYEETGNLYTKYSKDNQGVKIKYKYNKLNALDITDDYPNSRNKVVK LSLKPYPYFDVYLDNGVYKFTVKNLDVIKKENYEVNSKCYEAKKLLKISNQ AEFIASFYKNDLIKINGELYRVIGVNNSTRNIVELNMIDITYREYLENMNDKRP PHIHTIASKTQSIKKYSTDILGNLYEVKSKKHPQIIKKG	N580A	H557A	D10A
cCas9-v21	Staphylococcus aureus	MKRNYILGLDIGITSVGYGIIYETRDVIDAGVRLFEANVENNEGRRSKGA RRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGSLQKLEEEFSA ALLHLAKRRGVHNVNEEEDTGNELSTKEQISRNKALEEKYVAELQLERLKK DGEVRGSINRFKTSDYVKEAKQLLKVKAYHQLDQSFIDTYIDLLETRRTYYE GPGEPSFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTYQSSEDIQEELTNLSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPPKV DLSQQKEIPTTLVDDFILSPVVKRSFIQSIKVINAIKKYGLPNDIIELAREKNSK	N580A	H557A	D10A

		DAQKMINEMQKRNQRTNERIEIIRTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIIPRSVSFDNSFNKNVVKQEENSKGNRTPFQ YLSSSDSKISYETFKKHILNLAGKGGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHHAEDALIIANADFIKKEWKLDKAKKVMENQMFEKQAESMPETEIQ EYKEIFITPHQIKHKDFDKYKSHRVDKKNRKLINDTLSTRKDDKGNLTV NNLNGLYDKDNDKLLINKSPEKLLMYHHDPTQYQKLIMEQYGDENP LYKYYEETGNLYTKYSKDNQGPVKKIKYYGNKLNALHDITDDYPNSRNKVVK LSLKPYRFDVYLDNGVYKFVTVKNLDVIKENYYEVNSKCYEAKKLKISNQ AEFIASFYKNDLIKINGELYRVIGVNSDDRNIIELNMIDITYREYLENMNDKRP PHIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIKKG			
cCas9-v42	Staphylococcus aureus	MKRNILGLDIGITSVGYGIIDYETRDVIDAGVRLFEANVENNEGRRSKRGA RRLKRRRRHRIQRVKLLFDYNLLTDHSELGINPYEARVKGLSQKLEEEFSA ALLHLAKRRGVHNVNEVEEDTGNELSTKEQJSRNKALEEKYVAELQLERLKK DGEVRSINRFKTSDYVKEAKQLLVQKAYHQLDQSFIDTYIDLETRRTYE GPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYAYNADLYNALNDLN NLVITRDENEKLEYEKFQIENVFQKKKPTLKQIAKEILVNEEDIKGYRVST GKPEFTNLKVYHDIKDITARKEIENAEELLDQIAKILTYQSSEDIQEELTNLSE LTQEEIEQISNLKGYTGTHNLSKAINLILDELWHTNDNQIAIFNRLKLVPKV DLSQQKEIPTTLVDLSPVVKRSFIQSKVINAIKKYGLPNDIHELAREKNSK DAQKMINEMQKRNQRTNERIEIIRTGKENAKYLIEKIKLHDMQEGKCLYS LEAIPLEDLLNPNFNYEVDHIIIPRSVSFDNSFNKNVVKQEENSKGNRTPFQ YLSSSDSKISYETFKKHILNLAGKGGRISKTKKEYLLEERDINRFSVQKDFINRNL VDTRYATRGLMNLRSYFRVNNLDVVKVSINGGFTSFLRRKWFKKERNKG YKHHAEDALIIANADFIKKEWKLDKAKKVMENQMFEKQAESMPETEIQ EYKEIFITPHQIKHKDFDKYKSHRVDKKNRKLINDTLSTRKDDKGNLTV NNLNGLYDKDNDKLLINKSPEKLLMYHHDPTQYQKLIMEQYGDENP LYKYYEETGNLYTKYSKDNQGPVKKIKYYGNKLNALHDITDDYPNSRNKVVK LSLKPYRFDVYLDNGVYKFVTVKNLDVIKENYYEVNSKCYEAKKLKISNQ AEFIASFYKNDLIKINGELYRVIGVNNRNKIELNMIDITYREYLENMNDKRP PHIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQIKKG	N580A	H557A	D10A
CdiCas9	Corynebacterium diphtheriae	MKYHVGIDVGTFSVGLAAIEVDDAGMPIKTLISLVSHIHDSGLDPDEIKSAVT RLASSGIARRRRLRRRRRLQQLDKFIQRQGWVPIELEDYSDPLYPWKVR AELAASYIADEKERGEKLSVALRHIARHRGWRNPYAKVSSLYLDPGSPDAFK AIREIKRASGQPPPETATVGQMVTLCELGTLKLRGEGGVLSARLQQSDYAR EIQEICRMQEGQELRYKIIDVFAAESPKGSASSRVGKDPLQPGKNRALKAS DAFQRYRIALIGNLRVRVDGEKRLSVEKNLVFDHLVNLTPKKEPEWVTIA EILGIDRGQLIGTATMTDDGERAGARPPTHDTNRSIVNSRIAPLVDWWKTA SALEQHAMVKALESNAEVDDFSPEGAKVQAFADLDDVHAKLDSLHLPV GRAAYSEDTLVRRLRMLSDGVLDYARLQEFGEPSWTPPTPRIGEPVGNP AVDRVLKTVSRWLESATKTWGAPERVIIHVREGFVTEKAREMDGDMRR RAARNAKLFQEMQEKLNVQKPSRADLWRYQSVQRQNCQAYCGSPITF SNSEMDHIVPRAGQGSTNTRENLVAVCHRCNQSKGNTPFIAWAKNTSIEG VSVKEAVERTRHWVTDGMRSTDFFKFTKAVVERFQRATMDEIDARSME SVAWMANELRSRVAQHAFSHGTTVRVYRGLTAEARRASGISGKLKFFDGV GKSRLDRRHHAIDAAVIAFTSDYVAETLAVRSNLKQSQAHREQAPQWREFT GKDAEHRAAWRVWCQKMEKLSALLTEDLRDRVVVMSNVRLRLGNGSA HKETIGKLSKVLSSQLSVSDIDKASSEALWCALTREPFGDPKEGLPANPERHI RVNGTHVYAGDNIGLFPVSAGSIALRGGYALGSSFFHARVYKITSGKKPAF AMLRVYTIDLLPYRNQDLFSVELKPQTMMSMRQAEKKLRDALATGNAEYLG WLVVDDELVDVTSKIATDQVKAELGTIRRWVVDGFFSPSKLRLRPLQM SKEGIKESAPELSKIIDRPGWLPVAVNKLFSGDNVTVVRRDSLGRVRLESTAH LPVTWKVQ	N597A	H573A	D8A
CjeCas9	Campylobacter jejuni	MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRLARSA RKRLARRKARLNHLKHLIANEFKLNIEDYQSFDESLAKAYKGLISPYELRFR LNELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAIKAIKQNEEKLANYSQ VGEYLYKEYFQKFKEKNSKEFTNVRNKKESYERCIASFLKDELKLIKQREFG FSFSKKFEEVLSVAFYKRAKDFSHLVGNCSTFTDEKRAPKNSPLAFMFVAL TRIINLLNNLKNTEGILYTKDDLNALLNEVLKNGTLTYKQTKKLLGLSDDYEFK	N582A	H559A	D8A

		GEKGYTIEFKYKEFIKALGEHNLSQDDLNEIAKDITLIKDEIKLKKALAKYDLN QNDQDSLSKLEFKDHLNLSFKALKLVTPMLLEGKKYDEACNELNLKVAINEDK KDFLPAPNETYYKDEVNTPVVLRAIKEYRKVLNALLKKYGVHKNIELAREVG KNHSQRAKIEKEQNENYKAKKDAELECEKGLKINSKNILKRLFKKEQKFCAY SGEKISDLQDEKMLEIDHIYPYSRFSDDSYMKNVLVFTKQNKQEKLNQTPFE AFGNDSSAKWQKIEVLAKNLPTKKQKRIIDKNYKDEQKQNFKDRNLNDTRYI ARLVNLYTKDYLDLPLSDDENTKLNNTQKGSKVHVEAKSGMLTSALRHTW GFSAKDRNNHLHHAIDAVIIAYANNISIVKAFSDFKKEQESNSAELYAKKISELD YKNKRKFFEPFSGFRQKVLDKIDEIFVSKPERKKPSGALHEETFREKEEFYQSY GGKEGVLKALELKGIRKVNKIVKNGDMFRVDIFKHKTNKFYAVPIYTMDF ALKVLPNKAVARSSKKGEIKDWILMDENYEFCSLYKDSLIIQTKDMQPEFV YYNAFTSSTVSLVSKHDNKFETLSKNQKILFKNANEKVIASIGIQNLKVFEK YIVSALGEVTKAEFRQREDFKK			
GeoCas9	Geobacillus stearothermophilus	MRYKIGLDIGITSVGWAVMNLDPRIEDLGVRFIDRAENPQTGESLALPRRLA RSARRRLRRKRHLERIRRLVIREGILTKEELDKEEKHEIDVWQLRVEALDR KLNNDELARVLLHLAKRRGFSNRKRSRNSKNENSTMLKHIEENRAILSSYRTV GEMIVKDPKFALHKKRNKGENYNTIARDLLEIRLIFSKQREFGNMSCTEEF ENEYITIWASQRPVASKDDIEKKVGFCTFEPKEKRAPKATYTFQSFIWEHIN KLRLISPSGARGLTDEERRLLYEQAQKNKITYHDIRTLHLPPDDTYFGIVYDR GESRKQENENIRFLELDAYHQIRKAVDKYVGKGSSSFLPIDFTFGYALTFLKD DADIHSYLRNEYEQNGKRMPLANKVYDNELIEELLNLSFTKFGHLSLKALRS ILPYMEQGEVYSSACERAGYFTGPKKKQKTMLLNPPIANPVVMRALTQA RKVVNAIKKYGSPVSIHIELARDLSQTFDERRKTKEQDENRKNETAIRQL MEYGLTLNPTGHDIVKFLWSEQNGRCAYSQPIEIERLLEPGYVEVDHVIPY SRSLDDSYTNKVLVLTRENREKGNRIPAELVGVGTERWQQFETFLTNKQFS KKKRDRLRLHYDENEETEFKNRNLNDTRYISRFANFIREHLKFAESDDKQK VYTVNGRVTALHRSRWEFNKNREESDLHHAVDVAVVACTTPSDIAKVTAFY QRREQNKELAKKTEPHFPQPWPHFADELARLSKHPKESIKALNLGNYDDQ KLESQPVFVSRMPKRSVTGAHQETLRRYVGIDERSGKIQTVVTKLSEIKL DASGHFPMYGKESDPRTYEAIRQLLEHNNDPKAFQEPLYKPKNGEPGP VIRTVKIIDTKNQVIPLNDGKTVAYNSNIVRVDFEKDGKYCVPVYTMDIM KGILPNKAIEPNKPYSEWKEMTEDYTFRSLYPNDLIRIELPREKTVKTAAGEE INVKDVVYKYTIDSANGGLELISHDRFSLRGVGSRTLKRFEKYQVDVLGNI YKVRGEKRVGLASSAHSKPGKTIRPLQSTRD	N605A	H582A	D8A
iSpyMacCas9	Streptococcus spp.	MDKKYSIGLDIGITNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRNRYLQEIFSNEMAKVDDSSFFHRLSEESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSQKADLRILYALAH MIKFRGHFLIEGDLNPDNSDVDFLFIQLVQTYNQLFEENPINASGVDAKAILS ARLSKRKLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVCLKREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYPLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLFFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDQKSGKTILDFLKSDFGANR NFMQLIHDDSLTFKEDIQKQVSGQGDLSLHEHIANLAGSPAICKGILQTVKV VDELVKVMGRHKPENIVEMARENQTTQKQKNSRERMKRIEIGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDQVHVPQSF LKDDSIDNKVLTSDKNRGKSDNPVSEEVVKMKKNYWRQLLNAKLITQRKF DNLTKAERGGSLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVLSPQVQVNVKKEIQTVGQNGG LFDDNPKSPLEVTSPKLVPLKELNPKYGGYQKPTTAYPVLLITDTKQIPIV MNKKQFQNPVKFLRDRGYQVQVGNDFIKLPKYTLVDIGDGIKRLWASSKEI HKGNQLVSVSSKQILLYHAHHLSDLSNDYLNHNQVQFDVLFNEIISFSKKC KLGEHIQKIENVYSNKKNSASIEELAESFIKLGFTQLGATSPFNFLGVKLNQ	N863A	H840A	D10A

		KQYGGKKDYILPCTEGTLIRQISITGLYETRVDSLKIGEDSGSGSGSKRTADGSE FES			
NmeCas9	Neisseria meningitidis	MAAFKPNSINYILGLDIGIASVGWAMVEIDEEENPIRLIDLGVRFERAEVVK TGDSLAMARRLARSVRRLTRRRRAHRLRLTRRLKREGVLQAANFDENGLIKS LPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGETADKELG ALLKGVAGNAHALQTGDFRTPAELALNKFEKESGHIRNQRSYSHTFSRKDL QAEILLFEKQKEFGNPHVSGGLKEGIETLMTQRPALSGDAVQKMLGHCTF EPAEPKAAKNITYAERFIWLTCLNNLRILEQGSERPLTDTERATLMDEPYRKS KLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEMKAYHAISRALEKEGL KDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRKLDRIQPEILEALLKHISFDKFV QISLKALRRIVPLMEQKRYDEACAIEYGDHYGKKNTEEKIYLPPIPADEIRNP VVLRLSQARKVINGVVRRYGSPARIHIETAREVKSFKDRKEIEKRQREENRK DREKAAAKFREYFPNFVGEPSKDILKRLYEQQHGKCLYSGKEINLGRLEK GYVEIDHALPFSRTWDDSFNNKVLVLGSENQNGNQTPYEYFNGKDNSRE WQEFKARVETSRFPRSKQRILLQKFEDEGFKERNLNDTRYVNRFLCQFVA DRMRLTGKGGKRVFASNGQITNLLRGFWGLRKVRAENDRHHALDAVVVA CSTVAMQKQITRFVRYKEMNAFDGKTIDKETGEVLHQKTHFPQPWEFFAQ EVMIRVFGKPDGKPEFEEADTLEKRLTLAEKLSSRPEAVHEYVTPLFVSRAP NRKMSGQGHMETVKSARKLDEGVSVLRVPLTQLKLDLEKMNREREP YEALKARLEAHKDDPAKAFAPFYKYDKAGNRTQQVKAVRVEQVQKTGVV VRNHNGIADNATMVRVDVFEKGDYKYLVIYSWQVAKGILPDRVAVQVKD EEDWQLIDDSFNFKFSLHPNDLVEVITKARMFGYFASCHRG TGNNIRIHD LDHKIGKNIGLEGIGVKTALSFKYQIDELGKEIRPCRLKKRPPVR	N611A	H588A	D16A
ScaCas9	Streptococcus canis	MEKKYSIGLDIGTNSVGWAVITDDYKVPSSKKFKVLGNTNRKSIKKNLMGALL FDSGETAEATRLKRTARRRYTRRKNRIRYLQEIFANEMAKLDDSFQRLSEESF LVEEDKKNERHPIFGNLADEVAYHRNYPTIYHLRKKLADSPEKADRLIYLALA HIIKFRGHFLIEGKLNANSDVAKLFYQLIQTYNQLFEESPLDEIEVDAKGILSA RLSKSRLEKLIIVFPNEKKNLFGNIIALALGLTPNFKSNFDLTEDAKLQLSKD TYDDDLDELLGQIGDQYADLFSAAKNLSDAILLSDILRSNEVTKAPLSASMV KRYDEHHQDLALLKTLVRQQFPEKYAEIFKDDTKNGYAGYVGIGIKHRKRTT KLATQEEFYKFIKPILEKMDGAEELAKLNRDDLRLKQRTFDNGSIPHQIHLKE LHAILRRQEEFYFPLKENREKIEKILTRIPYVVGPLARGNSRFALWTRKSEEA TPWNFEVVDKGASAQSFIERMTNFDEQLPNKKVLPKHSLLYEYFTVYNELT KVYVTERMRKPEFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSV EIIGVEDRFNASLGTYHDLKKIKDKDFLDNEENEDILEDIVLTLTFEDREMIEE RLKTYAHLFDDKVMKQLKRRHYTGWGRLSRKMINGIRDKQSGKTILDFLKS DGFSNRNFMQLIHDDSLTFKEEIEKAQVSGQGDLSLHEQIADLAGSPAIKKGIL QTVKIVDELVKVMGHKPNIVIAMARENQTTKGLQSSRERKKRIEIGIKELE SQILKENPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVHIVP QSFIKDDSIDNKVLTFRSVENRGKSDNVPSSEVVKMKNYWRQLLNAKLITQ RKFDNLTKAERGGSEADKAGFIKRLQVETRQITKHVARILDSRMNTKRDKN DKPIREVKVITLKSCLVDFRQDFQLYKVRDINNHYHHAHDAYLNAVVGTA LIKYPKLESEFVYGDYKYVDVRKMIKSEQIEGKATAKRFYSNIMNFFKTEVKL ANGEIRKRPLIETNGETGEVVWNKEKDFATVRKVLAMPQVNVVKKTEVQ TGFSGESILSKRESAKLIPRKKGWDRKYGGGGSPTVAYSILVAKVEKGAKKL KSVKVLVGITIMEKGSYKDPGFLEAKGYKDIKKELIFKLPKYSLEFELNGRRR MLASATELQKANELVLPQHLVRLLYYTQNISATTGSNNLGYIEQHREEFKEIF EKIIDFSEKYLKKNVNSNLKSSFDEQFAVSDSILLSNSFVSLKYTSFGASGGFT FLDLVKKQGRRLRYQTVTEVLDATLYQISITGLYETRTDLSQLGGD	N872A	H849A	D10A
ScaCas9- HiFi-Sc++	Streptococcus canis	MEKKYSIGLDIGTNSVGWAVITDDYKVPSSKKFKVLGNTNRKSIKKNLMGALL FDSGETAEATRLKRTARRRYTRRKNRIRYLQEIFANEMAKLDDSFQRLSEESF LVEEDKKNERHPIFGNLADEVAYHRNYPTIYHLRKKLADSPEKADRLIYLALA HIIKFRGHFLIEGKLNANSDVAKLFYQLIQTYNQLFEESPLDEIEVDAKGILSA RLSKSRLEKLIIVFPNEKKNLFGNIIALALGLTPNFKSNFDLTEDAKLQLSKD TYDDDLDELLGQIGDQYADLFSAAKNLSDAILLSDILRSNEVTKAPLSASMV KRYDEHHQDLALLKTLVRQQFPEKYAEIFKDDTKNGYAGYVGADKLRKRS GKLATEEFYKFIKPILEKMDGAEELAKLNRDDLRLKQRTFDNGSIPHQIHLK ELHAILRRQEEFYFPLKENREKIEKILTRIPYVVGPLARGNSRFALWTRKSEEA ITPWNFEVVDKGASAQSFIERMTNFDEQLPNKKVLPKHSLLYEYFTVYNEL	N872A	H849A	D10A

		TKVKYVTERMRKPEFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDS VEIIGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIE ERLKYAHLFDDKVMKQLKRRHYTGWGRLSRKMINGIRDQSGKTILDFLKS DGFSNANFMQLIHDDSLTFKEIEKAQVSGQGDSLHEQIADLAGSPAICKGIL QTVKIVDELVKVMGHPENIVIAMARENQTTTKGLQQSRERKKRIEEGIKELE SQLKENPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVDHIVP QSFIKDDSIDNKVLTNRSDNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQ RKFDNLTKAERGGSEADKAGFIKRQLVETRQITKHVARILDSRMNTKRDKN DKPIREVKVITLKSCLVSDFRKDFQLYKVRDINNYHHAHDAYLNAVVGTAIK KYPKLESEFVYGDYKVDVRKMIKSEQEI GKATAKRFFYSNIMNFFKTEVKL ANGEIRKRPLIETNGETGEVWVWNEKDFATVRKVLAMPQVNIVKKEVQTG GFSKESILSKRESAKLIPRKGWDTRKYGGGSPTVAYSILVVAKEGKAKKL KSVKVLVGITIMEKGSYKDPGFLEAKGYKDIKELIFKLPKYSLEFENGRRR MLASAKELQKANELVLPQHVLRLYYTQNISATTGSNNLGYIEQHREEFKEIF EKIIDFSEKYLKNKVNLSKSSFDEQFAVSDSILLSNSFVSLKYTSFGASGGFT FLDLVQGRRLRYQTVTEVLDTLIIYQSITGLYETRDLSQLGGD			
SpyCas9- 3var-NRRH	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRNRYLQEIFSNEMAKVDDSFHRLSEESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKLVDSTKADRLIYLALAH MIKFRGHFLIEGDLNPDNSVDKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRRLLENIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MVKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEE FYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGIIPHQIHLGELHAILRRQ GDFYPFLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EVDKGSASQSFIERMTNFDKNLPNEKVLPHKSHLLYEFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYA HLFDDKVMKQLKRLRYTGWGRLSRKLINGIRDQSGKTILDFLSDGFANRN FMQLIHDDSLTFKEDIQAQVSGQGDSLHEIANLAGSPAICKGILQTVKVV DELVKVMGGHPENIVIAMARENQTTQKGQKNSRERMKRIEEGKELGSQI LKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVDHIVPQSF LKDDSIDNKVLTNRSDNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQRKF DNLTAKAERGGSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVGTAIKKYPK LESEFVYGDYKVDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWVKGRDFATVRKVLSPQVNIVKKEVQTGGFSKES ILPKGNSDKLIARKDWDPKYGGGNSPTAAYSVLVVAKEGKSKKLKSVK ELLGITIMERSSEFNPIGFLEAKGYKEVKKDLIILPKYSLEFENGRKRMLAS AGVLHKGNELALPSKYVNFLYLASHYEKLGSPEDNEQKQLFVEQHKHYLDE IIEQISEFSKRVILADANLDKVL SAYNKHDKPIREQAENIIHLFTLTNLGVPA FKYFDTTIDKKRYTSTKEVLDTLIIQSITGLYETRDLSQLGGD	N863A	H840A	D10A
SpyCas9- 3var-NRTH	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRNRYLQEIFSNEMAKVDDSFHRLSEESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKLVDSTKADRLIYLALAH MIKFRGHFLIEGDLNPDNSVDKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRRLLENIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MVKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEE FYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGIIPHQIHLGELHAILRRQ GDFYPFLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EVDKGSASQSFIERMTNFDKNLPNEKVLPHKSHLLYEFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYA HLFDDKVMKQLKRLRYTGWGRLSRKLINGIRDQSGKTILDFLSDGFANRN FMQLIHDDSLTFKEDIQAQVSGQGDSLHEIANLAGSPAICKGILQTVKVV DELVKVMGGHPENIVIAMARENQTTQKGQKNSRERMKRIEEGKELGSQI LKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVDHIVPQSF LKDDSIDNKVLTNRSDNRGKSDNVPSEEVVKMKMNYWRQLLNAKLITQRKF	N863A	H840A	D10A

		DNLTKAERGGSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEIGKATAYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVSMPQVNIKKTEVQTGGFSKES ILPKGNSDKLIARKKDWDPKKYGGFNSPTVAYSVLVAKVEKGSKKLKSVK ELLGITIMERSSEKNIPIGFLEAKGYKEVKKDLIIPKYSFLENGRKRMLAS ASVLHKGNEALPSKYVNFYLAHYEKLKGSSEDNKQKQLFVEQHKHYLDEI IEQISEFSKRVLADANLDKVLAYNKHDKPIREQAENIIHLFTLTNLGASAAF KYFDTTIGRKYLTSTKEVLDTLIHQSIITGLYETRIDLSQLGGD			
SpyCas9- 3var-NRCH	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRKNRICYLQEIFSNEMAKVDDSFHRLSESL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHRRKKLVDSDDKADLRILYALAH MIKFRGHFLIEGDLNPDNSDVDFLFIQLVQTYNQLFEEENPINASGVDAKAILS ARLSKSRRLNLIQAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MVKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEE FYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPIHQIHLGELHAILRRQ GDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASQAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTIYHDLKIIKDKDFLDNEEDILEDIVLTTLFEDREMIEERLKYA HLFDDKVMKQLKRLRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR FMQLIHDDSLTFKEDIQKQVSGQGDLSHEHIANLAGSPAIIKKGILQTVKV DELVKVMGGHKKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQ LKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVHIVPQSF LKDDSIDNKVLRSDKNRGKSDNVPSSEVVKMKKNYWRQLNAKLITQRKF DNLTKAERGGSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEIGKATAYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVSMPQVNIKKTEVQTGGFSKES ILPKGNSDKLIARKKDWDPKKYGGFNSPTVAYSVLVAKVEKGSKKLKSVK ELLGITIMERSSEKNIPIGFLEAKGYKEVKKDLIIPKYSFLENGRKRMLAS AGVLQKGNELALPSKYVNFYLAHYEKLKGSPEDEKQKQLFVEQHKHYLDE IIEQISEFSKRVLADANLDKVLAYNKHDKPIREQAENIIHLFTLTNLGAPAA FKYFDTTINRKQYNTTKEVLDTLIHQSIITGLYETRIDLSQLGGD	N863A	H840A	D10A
SpyCas9- HF1	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRKNRICYLQEIFSNEMAKVDDSFHRLSESL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHRRKKLVDSDDKADLRILYALAH MIKFRGHFLIEGDLNPDNSDVDFLFIQLVQTYNQLFEEENPINASGVDAKAILS ARLSKSRRLNLIQAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPIHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFE EVVDKGASQAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTIYHDLKIIKDKDFLDNEEDILEDIVLTTLFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKQVSGQGDLSHEHIANLAGSPAIIKKGILQTVKV VDELVKVMGRHKKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVHIVPQSF LKDDSIDNKVLRSDKNRGKSDNVPSSEVVKMKKNYWRQLNAKLITQRKF DNLTKAERGGSELDAKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEIGKATAYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVSMPQVNIKKTEVQTGGFSKES ILPKRNSDKLIARKKDWDPKKYGGFNSPTVAYSVLVAKVEKGSKKLKSVKE LLGITIMERSSEKNIPIGFLEAKGYKEVKKDLIIPKYSFLENGRKRMLASA GELQKGNELALPSKYVNFYLAHYEKLKGSPEDEKQKQLFVEQHKHYLDEI	N863A	H840A	D10A

		EQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHFLTITNLGAPAAF KYFDTTIDRKRYTSTKEVL DATLIHQSI TGLYETRIDLSQLGGD			
SpyCas9- QQR1	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRKNRICYLQEIFS NEMAKVDDSFHRL EESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAH MIKFRGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPW NFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSHLLYEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTITLTFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIIKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIE EGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNKVLT RSDKNRGKSDNVPSEEVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSEL DKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKS KLVSDFRKDFQFYKVR EINNYHHA DAYLNAVVG TALIKKYPK LESEFVYGDYKVYDVRKMIKSEQIEGKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKEVQTGGFSKES ILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAKVEKGSKKLKSVKE LLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLASA RELQKGNELALPSKYVNFY LASHYEKLGSPEDNEQQLFVEQHKHYLDEII EQISEFSKRVLADAKLQDKVL SAYNKH RDKPIREQAENIIHFLTITNLGAPAAF KYFDTTFKQKQYRSTKEVL DATLIHQSI TGLYETRIDLSQLGGD	N863A	H840A	D10A
SpyCas9- SpG	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRKNRICYLQEIFS NEMAKVDDSFHRL EESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAH MIKFRGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPW NFE EVVDKGASAQSFIERMTNFDKNLPNEKVLPHKSHLLYEYFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTITLTFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIIKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIE EGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNKVLT RSDKNRGKSDNVPSEEVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSEL DKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKS KLVSDFRKDFQFYKVR EINNYHHA DAYLNAVVG TALIKKYPK LESEFVYGDYKVYDVRKMIKSEQIEGKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIVKKEVQTGGFSKES ILPKRNSDKLIARKKDWDPKKYGGFLWPTVAYSVLVAKVEKGSKKLKSVK ELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIILPKYSLFELENGRKRMLAS AKQLQKGNELALPSKYVNFY LASHYEKLGSPEDNEQQLFVEQHKHYLDE IIEQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHFLTITNLGAPAA FKYFDTTIDRKQYRSTKEVL DATLIHQSI TGLYETRIDLSQLGGD	N863A	H840A	D10A
SpyCas9- VQR	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRKNRICYLQEIFS NEMAKVDDSFHRL EESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLR LIYLALAH MIKFRGHFLIEGDLNPDNSDVKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRRL ENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLS	N863A	H840A	D10A

		KD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVDKGSASQSFIERMTNFDKNLPNEKVLPHKSHLLYEFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEEDILEDIVLTLTLFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIIKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNVKLTSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSELDDKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKYPK LESEFVYGDYKVDVRKMAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEI RKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIKKTEVQTGGFSKES ILPKRNSDKLIARKDWDPKKYGGFVSPTVAYSVLVAKVEKGSKKLKSVE LLGITIMERSSEKFNPIDLEAKGYKEVKDLIILPKYSLFELENGRKRMLASA GELQKGNELALPSKYVNFLYLASHYEKLGSPEDNEQQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHLFTLNLGAPAAF KYFDTTIDRKQYRSTKEVL DATLIHQ SITGLYETRIDLSQLGGD			
SpyCas9- VRER	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL EESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTKADRLIYLALAH MIKFRGHFLIEGDLNPDNSVDKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDAKLQLS KD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQ EDFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFE EVDKGSASQSFIERMTNFDKNLPNEKVLPHKSHLLYEFTVYNELTKVKYVTE GMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEEDILEDIVLTLTLFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANR NFMQLIHDDSLTFKEDIQKAQVSGQDLSLHEHIANLAGSPAIIKGILQTVKV VDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQ ILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDINRLSDYDVDHIVPQSF LKDDSIDNVKLTSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKF DNLTKAERGGSELDDKAGFIKRLVETRQITKHVAQILD SRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKVRINNYHHAHDAYLNAVVG TALIKKYPK LESEFVYGDYKVDVRKMAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEI RKRPLIETNGETGEIVWDKGRDFATVRKVL SMPQVNIKKTEVQTGGFSKES ILPKRNSDKLIARKDWDPKKYGGFVSPTVAYSVLVAKVEKGSKKLKSVE LLGITIMERSSEKFNPIDLEAKGYKEVKDLIILPKYSLFELENGRKRMLASA RELQKGNELALPSKYVNFLYLASHYEKLGSPEDNEQQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVL SAYNKH RDKPIREQAENIIHLFTLNLGAPAAF KYFDTTIDRKEYRSTKEVL DATLIHQ SITGLYETRIDLSQLGGD	N863A	H840A	D10A
SpyCas9- xCas	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL EESFL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTKADRLIYLALAH MIKFRGHFLIEGDLNPDNSVDKLFQILVQTYNQLFEENPINASGVDAKAILS ARLSKSRLENLIAQLPGEKKNGLFGNLIASLGLTPNFKSNFDLAEDTKLQLS KD TYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKLYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGIIPHQIHLGELHAILRRQE DFYFPLKDNREKIEKILTRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEK VVDKGSASQSFIERMTNFDKNLPNEKVLPHKSHLLYEFTVYNELTKVKYVTE GMRKPAFLSGDQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEEDILEDIVLTLTLFEDREMIEERLKYA	N863A	H840A	D10A

		HLFDDKVMKQLKRRRYTGWGRSLRKLINGIRDQKSGKTILDFLKSDFANR NFIQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLAGSPAIAKKGILQTVKVV DELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQI LKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVDHIVPQSF LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRF DNLTKAERGGSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKREINNYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKES ILPKRNSDKLIARKKDWDPKKGFGFSPVAVSVLVAKVEKGSKKLKSVKE LLGITIMERSSEFNPIDFLEAKGYEVKKDLIIKPKYSFELENGRRKMLASA GVLQKGNELALPSKYVNFYLAHYEKLKGPEDNEQKQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVLAYSYNKHROKPIREQAENIIHLFTLTNLGAPAAF KYFDTTIDRKRYTSTKEVLDTLHQSITGLYETRIDLSQLGGD			
SpyCas9- xCas-NG	Streptococcus pyogenes	MDKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFVLGNTDRHSIKKNLIGALLF DSGETAEATRLKRTARRRYTRRRNRYCQLQEIFSNEMAKVDDSFHRLSESL VEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRILYALAH MIKFRGHFLIEGDLNPDNSVDKLIQVLVQTYNQLFEEPNINASGVDAKAILS ARLSKSRLENLIAQLPGEKKNGLFNGLIASLGLTPNFKSNFDLAEDTKLQLS KDTYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS MIKLYDEHHQDLTLKALVRQQLPEKYEIFDQSKNGYAGYIDGGASQEEF YKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGIIPHQJHLGELHAILRRQE DFYPFLKDNREKIEKILTRIPYVVGPLARGNSRFAWMTRKSEETITPWNFEK VVDKGASAQSFIERMTNFDKNLPNEKVLPHKSLLEYFTVYNELTKVKYVTE GMRKPAFLSGDQKKAIVDILLFTNRKVTYKQLKEDYFKKIECFDSVEISGVED RFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLTFEDREMIEERLKYA HLFDDKVMKQLKRRRYTGWGRSLRKLINGIRDQKSGKTILDFLKSDFANR NFIQLIHDDSLTFKEDIQKAQVSGQGDLSHEHIANLAGSPAIAKKGILQTVKVV DELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMKRIEIGIKELGSQI LKEHPVENTQLQNEKLYLYLQNGRDMYVDQELINRLSDYDVDHIVPQSF LKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRF DNLTKAERGGSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLI REVKVITLKSCLVSDFRKDFQFYKREINNYHHAHDAYLNAVVGTAIIKKYPK LESEFVYGDYKVDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEI RRRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKES IRPKRNSDKLIARKKDWDPKKGFGFSPVAVSVLVAKVEKGSKKLKSVKE LLGITIMERSSEFNPIDFLEAKGYEVKKDLIIKPKYSFELENGRRKMLASA RFLQKGNELALPSKYVNFYLAHYEKLKGPEDNEQKQLFVEQHKHYLDEII EQISEFSKRVLADANLDKVLAYSYNKHROKPIREQAENIIHLFTLTNLGAPRAF KYFDTTIDRKRYTSTKEVLDTLHQSITGLYETRIDLSQLGGD	N863A	H840A	D10A
St1Cas9- CNRZ1066	Streptococcus thermophilus	MSDLVLGLDIGISVGVGILNKVTGEIHKNSRIFPAAQAENNLVRRTRNRQG RRLARRKKHRRVRLNRLFEEGLITDFTKISINLNPYQLRVKGLTDELSNEELFI ALKNMVKHRGISYLDASDDGNSSVGDYQVKNESKQLETCTPGGQIQLER YQTYGQLRGDFTVEKDGGKHRLINVFTSAYRSEALRLQTQQEFNPQITDEF INRYLEILTGRKYYHGPNGEKSRDYGRYRTSGETLDNIFGILIGKCTFPDEF RAAKASYTAQEFNLLNDLNLNLTVPETETKLSKEQNQJINYYKNEKAMGPAK LFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKLTLETLDIEQMDRET DKLAYVLTNTEREGIQEALEHEFADGSFSQKQVDELVQFRKANSSIFGKGW HNFSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEIY NPVVAKSVRQAIKIVNAAIKEYGDFDNIVIAMARETNEDDEKKAQIKQKAN KDEKDAAMLKAANQYNGKAELPHSVFHHGKQLATKIRLWHQGERCLYT GKTISIHDNLNNSNQFEVDHILPSITFDDSLANKVLVYATANQEKGQRTPYQ ALDSMDDAWSFRELKAFVRESKTLSENKKKEYLLTEEDISKFDVRKKFIERNLV DTRYASRVVLNALQEHFRAHKIDTKVSVVRGQFTSQRRLHWGIEKTRDTYH HHAVDALIAASSQLNLWKKQKNTLVSYSEEQLLDIETGELISDDEYKESVFKA PYQHFDVTLKSKEFEDSILFSYQVDSKFNKISDATIYATRAKVGDKKDET YVLGKIKDIYTDGQYDAFMKIYKDKSKFLMYRHPDPTFEKVIPILENYPNK QMNEKGEVPCNPFLKYKEEHGYIRKYSKKGNGPEIKSLKYDYSKLLGNPIDI TPENSKNKVVLQSLKPWRDVFYFNKATGKYEILGLKYADLQFEKGTGTYKIS	N622A	H599A	D9A

		QEKYNDIKKKEGVDSSEFKFTLYKNDLLLVKDTETKEQQLFRFLSRTLPKQK HYVELKPYDKQKFEGGEALIKVLGNVANGGQCIKGLAKSNISYKVRTDVLG NQHIKNEGDKPKLDF			
St1Cas9- LMG1831	Streptococcus thermophilus	MSDLVLGLDIGISVGVGILNKVTGEIHKNSRIFPAAQAENNLVRRTRNRQG RRLARRKKHRRVRLNRLFEEESGLITDFTKISINLNPYQLRVKGLTDELSNEELFI ALKNMVKHKGISYLDASDDGNSSVGDYAQIVKENSQLETKTPGQIQLER YQTYGQLRGDFTVEKDGGKHRLINVFTSAYRSEALRLQTQQEFNPQITDEF INRYLEILTGRKYYHGPNGEKSRTDYGRYRTSGETLDNIFGILIGKCTFYPDF RAAKASYTAQEFNLLNDLNNLTVPTETKLSKEQKNQIINYVKNEKAMGPAK LFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKTLETLDIEQMDRETL DKLAYVLTNTEREGIQEALEHEFADGSFSQKQVDELVQFRKANSSIFGKGW HNFSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEEIY NPVVAKSVRQAIVNAAIKEYGDFDNIVIAMARETNEDDEKKAQIKQKAN KDEKDAAMLKAANQYNGKAELPHSVFHGHKQLATKIRLWHQQGERCLYT GKTISIHDLLNNSNQFEVDHILPLSITFDDSLANKVLVYATANQKEGQRTPYQ ALDSMDDAWSFRELFKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNLV DTRYASRVVLNALQEHFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDTH HHAVDALIAASSQLNLWKKQKNTLVSYSEEQLDIETGELISDDEYKESVFA PYQHFDVTLKSKEFEDSILFSYQVDSKFNRKISDATIYATRAKVGDKKDET YVLGKIKDIYTDQGYDAFMKIYKDKSKFLMYRHPDQTFEKVIEPILENYPNK QMNEKGKEVPCNPFKYKEEHGYIRKYSKKGNGPEIKSLKYDSDLGNPIDI TPENSKNKVVLQSLKPWRTDVYFNKNTGKYEILGLKYADLQFEKKTGTYSISK EKYNGIMKEEGVDSSEFKFTLYKNDLLLVKDTETKEQQLFRFLSRTMPNVK YYVELKPYSKDKFEKNESLIEILGSADKSGRCIKGLGKSNISYKVRTDVLGNQH IKNEGDKPKLDF	N622A	H599A	D9A
St1Cas9- MTH17CL3 96	Streptococcus thermophilus	MSDLVLGLDIGISVGVGILNKVTGEIHKNSRIFPAAQAENNLVRRTRNRQG RRLARRKKHRRVRLNRLFEEESGLITDFTKISINLNPYQLRVKGLTDELSNEELFI ALKNMVKHKGISYLDASDDGNSSVGDYAQIVKENSQLETKTPGQIQLER YQTYGQLRGDFTVEKDGGKHRLINVFTSAYRSEALRLQTQQEFNPQITDEF INRYLEILTGRKYYHGPNGEKSRTDYGRYRTSGETLDNIFGILIGKCTFYPDF RAAKASYTAQEFNLLNDLNNLTVPTETKLSKEQKNQIINYVKNEKAMGPAK LFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKTLETLDIEQMDRETL DKLAYVLTNTEREGIQEALEHEFADGSFSQKQVDELVQFRKANSSIFGKGW HNFSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEEIY NPVVAKSVRQAIVNAAIKEYGDFDNIVIAMARETNEDDEKKAQIKQKAN KDEKDAAMLKAANQYNGKAELPHSVFHGHKQLATKIRLWHQQGERCLYT GKTISIHDLLNNSNQFEVDHILPLSITFDDSLANKVLVYATANQKEGQRTPYQ ALDSMDDAWSFRELFKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNLV DTRYASRVVLNALQEHFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDTH HHAVDALIAASSQLNLWKKQKNTLVSYSEDQLDIETGELISDDEYKESVFA APYQHFDVTLKSKEFEDSILFSYQVDSKFNRKISDATIYATRAKVGDKKADE TYVLGKIKDIYTDQGYDAFMKIYKDKSKFLMYRHPDQTFEKVIEPILENYPN KQINEKGKEVPCNPFKYKEEHGYIRKYSKKGNGPEIKSLKYDSDLGNHIDIT PKDSNNKVVVLQSLKPWRTDVYFNKNTGKYEILGLKYSDMQFEKGTGKYSISK EQYENIKVREGVDENSEFKFTLYKNDLLLDSENGEQILLRFTSRNDTSKHYV ELKPYNRQKFEGSEYLIKSLGTAVAKGGQCIKGLGKSNISYKVRTDVLGNQHII KNEGDKPKLDF	N622A	H599A	D9A
St1Cas9- TH1477	Streptococcus thermophilus	MSDLVLGLDIGISVGVGILNKVTGEIHKNSRIFPAAQAENNLVRRTRNRQG RRLARRKKHRRVRLNRLFEEESGLITDFTKISINLNPYQLRVKGLTDELSNEELFI ALKNMVKHKGISYLDASDDGNSSVGDYAQIVKENSQLETKTPGQIQLER YQTYGQLRGDFTVEKDGGKHRLINVFTSAYRSEALRLQTQQEFNPQITDEF INRYLEILTGRKYYHGPNGEKSRTDYGRYRTSGETLDNIFGILIGKCTFYPDF RAAKASYTAQEFNLLNDLNNLTVPTETKLSKEQKNQIINYVKNEKAMGPAK LFKYIAKLLSCDVADIKGYRIDKSGKAEIHTFEAYRKMKTLETLDIEQMDRETL DKLAYVLTNTEREGIQEALEHEFADGSFSQKQVDELVQFRKANSSIFGKGW HNFSVKLMMELIPELYETSEEQMTILTRLGKQKTTSSSNKTKYIDEKLLTEEIY NPVVAKSVRQAIVNAAIKEYGDFDNIVIAMARETNEDDEKKAQIKQKAN KDEKDAAMLKAANQYNGKAELPHSVFHGHKQLATKIRLWHQQGERCLYT GKTISIHDLLNNSNQFEVDHILPLSITFDDSLANKVLVYATANQKEGQRTPYQ	N622A	H599A	D9A

		ALDSMDDAWSFRELKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNLV DTRYASRVVNLALQEHFRAHKIDTKVSVVRGQFTSQLRHGWGIEKTRDTH HHAVDALIIAASSQLNLWKKQKNTLVSYSEDQLLDIETGELISDDEYKESVFK APYQHFDVTLKSKEFEDSILFSYQVDSKFNKISDATIYATRQAKVGDKADE TYVLGKIKDIYTDQDGYDAFMKIYKKDKSKFLMYRHPQTFEKVIEPILENYPN KQINEKGKEVPCNPFLKYKEEHGYIRKYSKKGNGPEIKSLKYDSKLGHNHIDIT PKDSNNKVVLSLKPWRTDVYFNKNTGKYEILGKYSDMQFEKGTGKYSISK EQYENIKVREGVDENSEFKFTLYKNDLLLLKDSERGEQILLRFTSRNDTSKHYY ELKPYNRQKFEGSEYLIKSLGTVVKGGRCIKGLGKSNISYKVRTDVLGNQHIK NEGDKPKLDF			
sRGN3.1	Staphylococcus spp.	MNQKFILGLDIGITSVGYGLIDYETKNIIDAGVRLFPEANVENNEGRRSKRGS RRLKRRRIHRLERVKLLLEYDLINKEQIPTSNPNPYQIRVKGLSEILSKDELAIAL LHLAKRRGIHNVDAADKEETASDSLSTKDQINKNAKFLESRYVCELQKERLE NEGHRVGVENRFLTKDIVREAKIIDTQMYYPEIDETFKEKYISLVETRREYF EGPGQGSPFGWNGDLKKWYEMLMGHCTYFPQELRSVKYAYSADLFNALN DLNLLIQRDNSEKLEYHEKYHIIENVFKQKKPTLQJAKEIGVNPEDIKGYRI TKSGTPEFTSFKLFHDLKKVVKDHAILDDIDLLNQIAELTIYQDKDSIVAEGLQ LEYLMSEADKQISELTGYTGTHSLKCMNMIIDELWHSSMNQMEVFTYL NMRPKKYLKGYQRIPTDMIDDAILSPVVKRTFIQSINVINKVIEKYGIPEDIIE LARENNSDRRKFINNLQKKNEATKRINEIIGQTGNQNAKRIVEKIRLHDQ QEGKCLYSLESIPLDLLNPNHYVDHIIIPRSVSFDNSYHNKVLVKQSENSK KSNLTPYQYFNSGSKLSYNQFKQHILNLSKSQDRISKKKEYLLEERDINKFE VQKEFINRNLVDTRYATRELTYLKAYFSANNMNVKVTINGSFTDYLRKV WFKKERNHGYKHAEDALIANADFLKENKKLAVNSVLEKPEIETKQLDI QVDSEDNYSEMFIIPKQVQDIKDFRNFKYSHRVDKPNRQLINDTLYSTRK DNSTYIVQTIKDIYAKDNTTLKKQFQKSPEKFLMYQHDPRTFEKLEVIMKQYA NEKNPLAKYHEETGEYLTYSKKNNGPIVKSLEYGNLGSGLDVTHTQFSST KKLVKLSIKNYRFDVYLTEKGYKFVTIAYLNVFKKDNYYYIPKDKYQELKEKKI KDTDDQFIASFYKNDLIKNGDLYKIIGVNSDDRNIIELDYDIKYDYCEINNI GEPRIKKTIGKKTESIEKFTTDVLGNLYLHSTEKAPQLIFKRGL	N585A	H562A	D10A
sRGN3.3	Staphylococcus spp.	MNQKFILGLDIGITSVGYGLIDYETKNIIDAGVRLFPEANVENNEGRRSKRGS RRLKRRRIHRLERVKLLLEYDLINKEQIPTSNPNPYQIRVKGLSEILSKDELAIAL LHLAKRRGIHNVDAADKEETASDSLSTKDQINKNAKFLESRYVCELQKERLE NEGHRVGVENRFLTKDIVREAKIIDTQMYYPEIDETFKEKYISLVETRREYF EGPGQGSPFGWNGDLKKWYEMLMGHCTYFPQELRSVKYAYSADLFNALN DLNLLIQRDNSEKLEYHEKYHIIENVFKQKKPTLQJAKEIGVNPEDIKGYRI TKSGTPEFTSFKLFHDLKKVVKDHAILDDIDLLNQIAELTIYQDKDSIVAEGLQ LEYLMSEADKQISELTGYTGTHSLKCMNMIIDELWHSSMNQMEVFTYL NMRPKKYLKGYQRIPTDMIDDAILSPVVKRTFIQSINVINKVIEKYGIPEDIIE LARENNSDRRKFINNLQKKNEATKRINEIIGQTGNQNAKRIVEKIRLHDQ QEGKCLYSLESIPLDLLNPNHYVDHIIIPRSVSFDNSYHNKVLVKQSENSK KSNLTPYQYFNSGSKLSYNQFKQHILNLSKSQDRISKKKEYLLEERDINKFE VQKEFINRNLVDTRYATRELTYLKAYFSANNMDVKVTINGSFTNHLRKV WRFDKYRNHGYKHAEDALIANADFLKENKKLQNTNKILEKPTIENNTKK VTVEKEEDYNVFEPTKLVEDIKQYRDYKFSHRVDKPNRQLINDTLYSTRM KDEHDYIVQITDIYKGDNTNLKKQFNKNPEKFLMYQNDPKTFEKLIIIMKQ YSDEKNPLAKYEEETGEYLTYSKKNNGPIVKIKLLGNKVGNNHLDVTNKYEN STKKLVKLSIKNYRFDVYLTEKGYKFVTIAYLNVFKKDNYYYIPKDKYQELKEKK KIKDTDQFIASFYKNDLIKNGDLYKIIGVNSDDRNIIELDYDIKYDYCEINNI GEPRIKKTIGKKTESIEKFTTDVLGNLYLHSTEKAPQLIFKRGL	N585A	H562A	D10A

In some embodiments, a Cas protein requires a protospacer adjacent motif (PAM) to be present in or adjacent to a target DNA sequence for the Cas protein to bind and/or function. In

some embodiments, the PAM is or comprises, from 5' to 3', NGG, YG, NNGRRT, NNNRRT, NGA, TYCV, TATV, NTTN, or NNNGATT, where N stands for any nucleotide, Y stands for C or T, R stands for A or G, and V stands for A or C or G. In some embodiments, a Cas protein is a protein listed in Table 3 or 4. In some embodiments, a Cas protein comprises one or more mutations altering its PAM. In some embodiments, a Cas protein comprises E1369R, E1449H, and R1556A mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises E782K, N968K, and R1015H mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises D1135V, R1335Q, and T1337R mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises S542R and K607R mutations or analogous substitutions to the amino acids corresponding to said positions. In some embodiments, a Cas protein comprises S542R, K548V, and N552R mutations or analogous substitutions to the amino acids corresponding to said positions. Exemplary advances in the engineering of Cas enzymes to recognize altered PAM sequences are reviewed in Collias et al Nature Communications 12:555 (2021), incorporated herein by reference in its entirety.

In some embodiments, the Cas protein is catalytically active and cuts one or both strands of the target DNA site. In some embodiments, cutting the target DNA site is followed by formation of an alteration, e.g., an insertion or deletion, e.g., by the cellular repair machinery.

In some embodiments, the Cas protein is modified to deactivate or partially deactivate the nuclease, e.g., nuclease-deficient Cas9. Whereas wild-type Cas9 generates double-strand breaks (DSBs) at specific DNA sequences targeted by a gRNA, a number of CRISPR endonucleases having modified functionalities are available, for example: a “nickase” version of Cas9 that has been partially deactivated generates only a single-strand break; a catalytically inactive Cas9 (“dCas9”) does not cut target DNA. In some embodiments, dCas9 binding to a DNA sequence may interfere with transcription at that site by steric hindrance. In some embodiments, dCas9 binding to an anchor sequence may interfere with (e.g., decrease or prevent) genomic complex (e.g., ASMC) formation and/or maintenance. In some embodiments, a DNA-binding domain comprises a catalytically inactive Cas9, e.g., dCas9. Many catalytically inactive Cas9 proteins are known in the art. In some embodiments, dCas9 comprises mutations in each endonuclease domain of the Cas protein, e.g., D10A and H840A or N863A mutations. In some embodiments, a

catalytically inactive or partially inactive CRISPR/Cas domain comprises a Cas protein comprising one or more mutations, e.g., one or more of the mutations listed in Table 3. In some embodiments, a Cas protein described on a given row of Table 3 comprises one, two, three, or all of the mutations listed in the same row of Table 3. In some embodiments, a Cas protein, e.g., not
5 described in Table 3, comprises one, two, three, or all of the mutations listed in a row of Table 3 or a corresponding mutation at a corresponding site in that Cas protein.

In some embodiments, a catalytically inactive, e.g., dCas9, or partially deactivated Cas9 protein comprises a D11 mutation (e.g., D11A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9
10 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a H969 mutation (e.g., H969A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a N995 mutation (e.g., N995A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive
15 Cas9 protein, e.g., dCas9, comprises mutations at one, two, or three of positions D11, H969, and N995 (e.g., D11A, H969A, and N995A mutations) or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a D10 mutation (e.g., a D10A mutation) or an analogous
20 substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a H557 mutation (e.g., a H557A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D10 mutation (e.g., a D10A mutation) and a H557 mutation (e.g., a H557A
25 mutation) or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a D839 mutation (e.g., a D839A mutation) or an analogous
substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a
30 H840 mutation (e.g., a H840A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g.,

dCas9, or partially deactivated Cas9 protein comprises a N863 mutation (e.g., a N863A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D10 mutation (e.g., D10A), a D839 mutation (e.g., D839A), a H840 mutation (e.g., H840A), and a N863 mutation
5 (e.g., N863A) or analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a E993 mutation (e.g., a E993A mutation) or an analogous substitution to the amino acid corresponding to said position.

In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially
10 deactivated Cas9 protein comprises a D917 mutation (e.g., a D917A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a a E1006 mutation (e.g., a E1006A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g.,
15 dCas9, or partially deactivated Cas9 protein comprises a D1255 mutation (e.g., a D1255A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D917 mutation (e.g., D917A), a E1006 mutation (e.g., E1006A), and a D1255 mutation (e.g., D1255A) or analogous substitutions to the amino acids corresponding to said positions.

20 In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a D16 mutation (e.g., a D16A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a D587 mutation (e.g., a D587A mutation) or an analogous substitution to the amino acid
25 corresponding to said position. In some embodiments, a partially deactivated Cas domain has nickase activity. In some embodiments, a partially deactivated Cas9 domain is a Cas9 nickase domain. In some embodiments, the catalytically inactive Cas domain or dead Cas domain produces no detectable double strand break formation. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or partially deactivated Cas9 protein comprises a H588
30 mutation (e.g., a H588A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, or

partially deactivated Cas9 protein comprises a N611 mutation (e.g., a N611A mutation) or an analogous substitution to the amino acid corresponding to said position. In some embodiments, a catalytically inactive Cas9 protein, e.g., dCas9, comprises a D16 mutation (e.g., D16A), a D587 mutation (e.g., D587A), a H588 mutation (e.g., H588A), and a N611 mutation (e.g., N611A) or
 5 analogous substitutions to the amino acids corresponding to said positions.

In some embodiments, a DNA-binding domain or endonuclease domain may comprise a Cas molecule comprising or linked (e.g., covalently) to a gRNA (e.g., a template nucleic acid, e.g., template RNA, comprising a gRNA).

In some embodiments, an endonuclease domain or DNA binding domain comprises a
 10 *Streptococcus pyogenes* Cas9 (SpCas9) or a functional fragment or variant thereof. In some embodiments, the endonuclease domain or DNA binding domain comprises a modified SpCas9. In embodiments, the modified SpCas9 comprises a modification that alters protospacer-adjacent motif (PAM) specificity. In embodiments, the PAM has specificity for the nucleic acid sequence 5'-NGT-3'. In embodiments, the modified SpCas9 comprises one or more amino acid
 15 substitutions, e.g., at one or more of positions L1111, D1135, G1218, E1219, A1322, of R1335, e.g., selected from L1111R, D1135V, G1218R, E1219F, A1322R, R1335V. In embodiments, the modified SpCas9 comprises the amino acid substitution T1337R and one or more additional amino acid substitutions, e.g., selected from L1111, D1135L, S1136R, G1218S, E1219V, D1332A, D1332S, D1332T, D1332V, D1332L, D1332K, D1332R, R1335Q, T1337, T1337L,
 20 T1337Q, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereto. In embodiments, the modified SpCas9 comprises: (i) one or more amino acid substitutions selected from D1135L, S1136R, G1218S, E1219V, A1322R, R1335Q, and T1337; and (ii) one or more amino acid substitutions selected from L1111R, G1218R, E1219F, D1332A, D1332S, D1332T, D1332V, D1332L,
 25 D1332K, D1332R, T1337L, T1337I, T1337V, T1337F, T1337S, T1337N, T1337K, T1337R, T1337H, T1337Q, and T1337M, or corresponding amino acid substitutions thereto.

In some embodiments, the endonuclease domain or DNA binding domain comprises a Cas domain, e.g., a Cas9 domain. In embodiments, the endonuclease domain or DNA binding domain comprises a nuclease-active Cas domain, a Cas nickase (nCas) domain, or a nuclease-
 30 inactive Cas (dCas) domain. In embodiments, the endonuclease domain or DNA binding domain comprises a nuclease-active Cas9 domain, a Cas9 nickase (nCas9) domain, or a nuclease-inactive

Cas9 (dCas9) domain. In some embodiments, the endonuclease domain or DNA binding domain comprises a Cas9 domain of Cas9 (e.g., dCas9 and nCas9), Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, or Cas12i. In some embodiments, the endonuclease domain or DNA binding domain comprises a Cas9 (e.g., dCas9 and nCas9),

5 Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, or Cas12i. In some embodiments, the endonuclease domain or DNA binding domain comprises an *S. pyogenes* or an *S. thermophilus* Cas9, or a functional fragment thereof. In some embodiments, the endonuclease domain or DNA binding domain comprises a Cas9 sequence, e.g., as described in Chylinski, Rhun, and Charpentier (2013) RNA Biology 10:5, 726-737;

10 incorporated herein by reference. In some embodiments, the endonuclease domain or DNA binding domain comprises the HNH nuclease subdomain and/or the RuvC1 subdomain of a Cas, e.g., Cas9, e.g., as described herein, or a variant thereof. In some embodiments, the endonuclease domain or DNA binding domain comprises Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, or Cas12i. In some embodiments,

15 the endonuclease domain or DNA binding domain comprises a Cas polypeptide (e.g., enzyme), or a functional fragment thereof. In embodiments, the Cas polypeptide (e.g., enzyme) is selected from Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas5d, Cas5t, Cas5h, Cas5a, Cas6, Cas7, Cas8, Cas8a, Cas8b, Cas8c, Cas9 (e.g., Csn1 or Csx12), Cas10, Cas10d, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, Csy1, Csy2, Csy3, Csy4,

20 Cse1, Cse2, Cse3, Cse4, Cse5e, Csc1, Csc2, Csa5, Csn1, Csn2, Csm1, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx1S, Csx11, Csf1, Csf2, CsO, Csf4, Csd1, Csd2, Cst1, Cst2, Csh1, Csh2, Csa1, Csa2, Csa3, Csa4, Csa5, Type II Cas effector proteins, Type V Cas effector proteins, Type VI Cas effector proteins, CARF, DinG, Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12b/C2c1,

25 Cas12c/C2c3, SpCas9(K855A), eSpCas9(1.1), SpCas9-HF1, hyper accurate Cas9 variant (HypaCas9), homologues thereof, modified or engineered versions thereof, and/or functional fragments thereof. In embodiments, the Cas9 comprises one or more substitutions, e.g., selected from H840A, D10A, P475A, W476A, N477A, D1125A, W1126A, and D1127A. In

embodiments, the Cas9 comprises one or more mutations at positions selected from: D10, G12,

30 G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987, e.g., one or more substitutions selected from D10A, G12A, G17A, E762A, H840A, N854A, N863A, H982A,

H983A, A984A, and/or D986A. In some embodiments, the endonuclease domain or DNA binding domain comprises a Cas (e.g., Cas9) sequence from *Corynebacterium ulcerans*, *Corynebacterium diphtheria*, *Spiroplasma syrphidicola*, *Prevotella intermedia*, *Spiroplasma taiwanense*, *Streptococcus iniae*, *Belliella baltica*, *Psychroflexus torquis*, *Streptococcus*
 5 *thermophilus*, *Listeria innocua*, *Campylobacter jejuni*, *Neisseria meningitidis*, *Streptococcus pyogenes*, or *Staphylococcus aureus*, or a fragment or variant thereof.

In some embodiments, the endonuclease domain or DNA binding domain comprises a CpfI domain, e.g., comprising one or more substitutions, e.g., at position D917, E1006A, D1255 or any combination thereof, e.g., selected from D917A, E1006A, D1255A, D917A/E1006A,
 10 D917A/D1255A, E1006A/D1255A, and D917A/E1006A/D1255A.

In some embodiments, the endonuclease domain or DNA binding domain comprises spCas9, spCas9-VRQR(SEQ ID NO: 4019), spCas9-VRER(SEQ ID NO: 4020), xCas9 (sp), saCas9, saCas9-KKH, spCas9-MQKSER(SEQ ID NO: 4021), spCas9-LRKIQK(SEQ ID NO: 4022), or spCas9-LRVSQL(SEQ ID NO: 4023).

15 In some embodiments, a gene modifying polypeptide has an endonuclease domain comprising a Cas9 nickase, e.g., Cas9 H840A. In embodiments, the Cas9 H840A has the following amino acid sequence:

Cas9 nickase (H840A):

20 DKKYSIGLDIGTNSVGWAVITDEYKVPSSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
 TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDKKHERHPIFGN
 IVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMIKFRGHFLIEGDLNPDNSDV
 DKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLFGNLI
 ALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLNLLAQIGDQYADLFLAAKNLSDAIL
 25 LSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG
 YIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAI
 LRRQEDFYPLKDNREKIEKILTFRIPIYVGPLARGNSRFAWMTRKSEETITPWNFEEVV
 DKGASAQSFIERMTNFDKNLPNEKVLPHSLLYEYFTVYNELTKVKYVTEGMRKPAFLS
 GEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKII
 30 KDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWG
 RLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSL

HEHIANLAGSPAIIKGILQTVKVVDDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRE
 RMKRIIEGIGELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDV
 DAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRK
 FDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVI
 5 TLKSKLVSDFRKDFQFYKvreINNYHHAHDAYLNAVVGtalIKKYPKLESEFVYGDYK
 VYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWD
 KGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGG
 FDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYKEVKK
 DLIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGPED
 10 NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL SAYNKHrdKPIREQAENIIHL
 FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQsITGLYETRIDLSQLGGD

In some embodiments, a gene modifying polypeptide comprises a dCas9 sequence
 comprising a D10A and/or H840A mutation, e.g., the following sequence:

15 SMDKKYSIGLAIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGET
 AEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPI
 FGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADRLIYLALAHMIKFRGHFLIEGDLNPDN
 SDVDKLFQILVQTYNQLFEENPINASGVDAKAILSARLSKSRLENLIAQLPGEKKNGLF
 20 GNLIASLGLTPNFKSNFDLAEDAKLQLSKDQYDDDLNLLAQIGDQYADLFLAAKNLS
 DAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNG
 YAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGE
 LHAILRRQEDFYFPLKDNREKIEKILTFRIPIYVVGPLARGNSRFAWMTRKSEETITPWNFE
 EVVDKGASAQSFIERMTNFDKNLPNEKVLPHSLLEYEFTVYNELTKVKYVTEGMRKP
 25 AFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDL
 LKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYT
 GWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFMLIHDDSLTFKEDIQKAQVSGQ
 GDSLHEHIANLAGSPAIIKGILQTVKVVDDELVKVMGRHKPENIVIEMARENQTTQKGQK
 NSRERMKRIIEGIGELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLS
 30 DYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLI
 TQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIR
 EVKVITLKSKLVSDFRKDFQFYKvreINNYHHAHDAYLNAVVGtalIKKYPKLESEFVY
 GDYKVYDVRKMIKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETG
 EIVWDKGRDFATVRKVLSPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
 35 KYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSSFENPIDFLEAKGYK
 EVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGPED
 NEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVL SAYNKHrdKPIREQAENIIHL
 FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVL DATLIHQsITGLYETRIDLSQLGGD
 (SEQ ID NO: 4007)

TAL Effectors and Zinc Finger Nucleases

In some embodiments, an endonuclease domain or DNA-binding domain comprises a TAL effector molecule. A TAL effector molecule, e.g., a TAL effector molecule that specifically binds a DNA sequence, typically comprises a plurality of TAL effector domains or fragments thereof, and optionally one or more additional portions of naturally occurring TAL effectors (e.g., N- and/or C-terminal of the plurality of TAL effector domains). Many TAL effectors are known to those of skill in the art and are commercially available, e.g., from Thermo Fisher Scientific.

Naturally occurring TALEs are natural effector proteins secreted by numerous species of bacterial pathogens including the plant pathogen *Xanthomonas* which modulates gene expression in host plants and facilitates bacterial colonization and survival. The specific binding of TAL effectors is based on a central repeat domain of tandemly arranged nearly identical repeats of typically 33 or 34 amino acids (the repeat-variable di-residues, RVD domain).

Members of the TAL effectors family differ mainly in the number and order of their repeats. The number of repeats typically ranges from 1.5 to 33.5 repeats and the C-terminal repeat is usually shorter in length (e.g., about 20 amino acids) and is generally referred to as a “half-repeat.” Each repeat of the TAL effector generally features a one-repeat-to-one-base-pair correlation with different repeat types exhibiting different base-pair specificity (one repeat recognizes one base-pair on the target gene sequence). Generally, the smaller the number of repeats, the weaker the protein-DNA interactions. A number of 6.5 repeats has been shown to be sufficient to activate transcription of a reporter gene (Scholze et al., 2010).

Repeat to repeat variations occur predominantly at amino acid positions 12 and 13, which have therefore been termed “hypervariable” and which are responsible for the specificity of the interaction with the target DNA promoter sequence, as shown in Table 5 listing exemplary repeat variable diresidues (RVD) and their correspondence to nucleic acid base targets.

Table 5 – RVDs and Nucleic Acid Base Specificity

Target	Possible RVD Amino Acid Combinations												
A	NI	NN	CI	HI	KI								
G	NN	GN	SN	VN	LN	DN	QN	EN	HN	RH	NK	AN	FN

C	HD	RD	KD	ND	AD								
T	NG	HG	VG	IG	EG	MG	YG	AA	EP	VA	QG	KG	RG

Accordingly, it is possible to modify the repeats of a TAL effector to target specific DNA sequences. Further studies have shown that the RVD NK can target G. Target sites of TAL effectors also tend to include a T flanking the 5' base targeted by the first repeat, but the exact mechanism of this recognition is not known. More than 113 TAL effector sequences are known to date. Non-limiting examples of TAL effectors from *Xanthomonas* include, Hax2, Hax3, Hax4, AvrXa7, AvrXa10 and AvrBs3.

Accordingly, the TAL effector domain of a TAL effector molecule described herein may be derived from a TAL effector from any bacterial species (e.g., *Xanthomonas* species such as the African strain of *Xanthomonas oryzae* pv. *Oryzae* (Yu et al. 2011), *Xanthomonas campestris* pv. *raphani* strain 756C and *Xanthomonas oryzae* pv. *oryzicola* strain BLS256 (Bogdanove et al. 2011). In some embodiments, the TAL effector domain comprises an RVD domain as well as flanking sequence(s) (sequences on the N-terminal and/or C-terminal side of the RVD domain) also from the naturally occurring TAL effector. It may comprise more or fewer repeats than the RVD of the naturally occurring TAL effector. The TAL effector molecule can be designed to target a given DNA sequence based on the above code and others known in the art. The number of TAL effector domains (e.g., repeats (monomers or modules)) and their specific sequence can be selected based on the desired DNA target sequence. For example, TAL effector domains, e.g., repeats, may be removed or added in order to suit a specific target sequence. In an embodiment, the TAL effector molecule of the present invention comprises between 6.5 and 33.5 TAL effector domains, e.g., repeats. In an embodiment, TAL effector molecule of the present invention comprises between 8 and 33.5 TAL effector domains, e.g., repeats, e.g., between 10 and 25 TAL effector domains, e.g., repeats, e.g., between 10 and 14 TAL effector domains, e.g., repeats.

In some embodiments, the TAL effector molecule comprises TAL effector domains that correspond to a perfect match to the DNA target sequence. In some embodiments, a mismatch between a repeat and a target base-pair on the DNA target sequence is permitted as long as it allows for the function of the polypeptide comprising the TAL effector molecule. In general, TALE binding is inversely correlated with the number of mismatches. In some embodiments,

the TAL effector molecule of a polypeptide of the present invention comprises no more than 7 mismatches, 6 mismatches, 5 mismatches, 4 mismatches, 3 mismatches, 2 mismatches, or 1 mismatch, and optionally no mismatch, with the target DNA sequence. Without wishing to be bound by theory, in general the smaller the number of TAL effector domains in the TAL effector molecule, the smaller the number of mismatches will be tolerated and still allow for the function of the polypeptide comprising the TAL effector molecule. The binding affinity is thought to depend on the sum of matching repeat-DNA combinations. For example, TAL effector molecules having 25 TAL effector domains or more may be able to tolerate up to 7 mismatches.

In addition to the TAL effector domains, the TAL effector molecule of the present invention may comprise additional sequences derived from a naturally occurring TAL effector. The length of the C-terminal and/or N-terminal sequence(s) included on each side of the TAL effector domain portion of the TAL effector molecule can vary and be selected by one skilled in the art, for example based on the studies of Zhang et al. (2011). Zhang et al., have characterized a number of C-terminal and N-terminal truncation mutants in Hax3 derived TAL-effector based proteins and have identified key elements, which contribute to optimal binding to the target sequence and thus activation of transcription. Generally, it was found that transcriptional activity is inversely correlated with the length of N-terminus. Regarding the C-terminus, an important element for DNA binding residues within the first 68 amino acids of the Hax 3 sequence was identified. Accordingly, in some embodiments, the first 68 amino acids on the C-terminal side of the TAL effector domains of the naturally occurring TAL effector is included in the TAL effector molecule. Accordingly, in an embodiment, a TAL effector molecule comprises 1) one or more TAL effector domains derived from a naturally occurring TAL effector; 2) at least 70, 80, 90, 100, 110, 120, 130, 140, 150, 170, 180, 190, 200, 220, 230, 240, 250, 260, 270, 280 or more amino acids from the naturally occurring TAL effector on the N-terminal side of the TAL effector domains; and/or 3) at least 68, 80, 90, 100, 110, 120, 130, 140, 150, 170, 180, 190, 200, 220, 230, 240, 250, 260 or more amino acids from the naturally occurring TAL effector on the C-terminal side of the TAL effector domains.

In some embodiments, an endonuclease domain or DNA-binding domain is or comprises a Zn finger molecule. A Zn finger molecule comprises a Zn finger protein, e.g., a naturally occurring Zn finger protein or engineered Zn finger protein, or fragment thereof. Many Zn

finger proteins are known to those of skill in the art and are commercially available, e.g., from Sigma-Aldrich.

In some embodiments, a Zn finger molecule comprises a non-naturally occurring Zn finger protein that is engineered to bind to a target DNA sequence of choice. See, for example, Beerli, et al. (2002) *Nature Biotechnol.* 20:135-141; Pabo, et al. (2001) *Ann. Rev. Biochem.* 70:313-340; Isalan, et al. (2001) *Nature Biotechnol.* 19:656-660; Segal, et al. (2001) *Curr. Opin. Biotechnol.* 12:632-637; Choo, et al. (2000) *Curr. Opin. Struct. Biol.* 10:411-416; U.S. Pat. Nos. 6,453,242; 6,534,261; 6,599,692; 6,503,717; 6,689,558; 7,030,215; 6,794,136; 7,067,317; 7,262,054; 7,070,934; 7,361,635; 7,253,273; and U.S. Patent Publication Nos. 2005/0064474; 2007/0218528; 2005/0267061, all incorporated herein by reference in their entireties.

An engineered Zn finger protein may have a novel binding specificity, compared to a naturally-occurring Zn finger protein. Engineering methods include, but are not limited to, rational design and various types of selection. Rational design includes, for example, using databases comprising triplet (or quadruplet) nucleotide sequences and individual Zn finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is associated with one or more amino acid sequences of zinc fingers which bind the particular triplet or quadruplet sequence. See, for example, U.S. Pat. Nos. 6,453,242 and 6,534,261, incorporated by reference herein in their entireties.

Exemplary selection methods, including phage display and two-hybrid systems, are disclosed in U.S. Pat. Nos. 5,789,538; 5,925,523; 6,007,988; 6,013,453; 6,410,248; 6,140,466; 6,200,759; and 6,242,568; as well as International Patent Publication Nos. WO 98/37186; WO 98/53057; WO 00/27878; and WO 01/88197 and GB 2,338,237. In addition, enhancement of binding specificity for zinc finger proteins has been described, for example, in International Patent Publication No. WO 02/077227.

In addition, as disclosed in these and other references, zinc finger domains and/or multi-fingered zinc finger proteins may be linked together using any suitable linker sequences, including for example, linkers of 5 or more amino acids in length. See, also, U.S. Pat. Nos. 6,479,626; 6,903,185; and 7,153,949 for exemplary linker sequences 6 or more amino acids in length. The proteins described herein may include any combination of suitable linkers between the individual zinc fingers of the protein. In addition, enhancement of binding specificity for zinc

finger binding domains has been described, for example, in co-owned International Patent Publication No. WO 02/077227.

Zn finger proteins and methods for design and construction of fusion proteins (and polynucleotides encoding same) are known to those of skill in the art and described in detail in
5 U.S. Pat. Nos. 6,140,0815; 789,538; 6,453,242; 6,534,261; 5,925,523; 6,007,988; 6,013,453; and 6,200,759; International Patent Publication Nos. WO 95/19431; WO 96/06166; WO 98/53057; WO 98/54311; WO 00/27878; WO 01/60970; WO 01/88197; WO 02/099084; WO 98/53058; WO 98/53059; WO 98/53060; WO 02/016536; and WO 03/016496.

In addition, as disclosed in these and other references, Zn finger proteins and/or multi-
10 fingered Zn finger proteins may be linked together, e.g., as a fusion protein, using any suitable linker sequences, including for example, linkers of 5 or more amino acids in length. See, also, U.S. Pat. Nos. 6,479,626; 6,903,185; and 7,153,949 for exemplary linker sequences 6 or more amino acids in length. The Zn finger molecules described herein may include any combination of suitable linkers between the individual zinc finger proteins and/or multi-fingered Zn finger
15 proteins of the Zn finger molecule.

In certain embodiments, the DNA-binding domain or endonuclease domain comprises a Zn finger molecule comprising an engineered zinc finger protein that binds (in a sequence-specific manner) to a target DNA sequence. In some embodiments, the Zn finger molecule comprises one Zn finger protein or fragment thereof. In other embodiments, the Zn finger
20 molecule comprises a plurality of Zn finger proteins (or fragments thereof), e.g., 2, 3, 4, 5, 6 or more Zn finger proteins (and optionally no more than 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 Zn finger proteins). In some embodiments, the Zn finger molecule comprises at least three Zn finger proteins. In some embodiments, the Zn finger molecule comprises four, five or six fingers. In some embodiments, the Zn finger molecule comprises 8, 9, 10, 11 or 12 fingers. In some
25 embodiments, a Zn finger molecule comprising three Zn finger proteins recognizes a target DNA sequence comprising 9 or 10 nucleotides. In some embodiments, a Zn finger molecule comprising four Zn finger proteins recognizes a target DNA sequence comprising 12 to 14 nucleotides. In some embodiments, a Zn finger molecule comprising six Zn finger proteins recognizes a target DNA sequence comprising 18 to 21 nucleotides.

30 In some embodiments, a Zn finger molecule comprises a two-handed Zn finger protein. Two handed zinc finger proteins are those proteins in which two clusters of zinc finger proteins

are separated by intervening amino acids so that the two zinc finger domains bind to two discontinuous target DNA sequences. An example of a two handed type of zinc finger binding protein is SIPI, where a cluster of four zinc finger proteins is located at the amino terminus of the protein and a cluster of three Zn finger proteins is located at the carboxyl terminus (see

5 Remade, et al. (1999) EMBO Journal 18(18):5073-5084). Each cluster of zinc fingers in these proteins is able to bind to a unique target sequence and the spacing between the two target sequences can comprise many nucleotides.

Linkers

In some embodiments, a gene modifying polypeptide may comprise a linker, e.g., a peptide linker, e.g., a linker as described in Table 6. In some embodiments, a gene modifying polypeptide comprises, in an N-terminal to C-terminal direction, a Cas domain (e.g., a Cas domain of Table 3), a linker of Table 6 (or a sequence having at least 70%, 80%, 85%, 90%, 95%, or 99% identity thereto), and an RT domain (e.g., an RT domain of Table 2). In some

10 embodiments, a gene modifying polypeptide comprises a flexible linker between the endonuclease and the RT domain, e.g., a linker comprising the amino acid sequence SGGSSGGSSGSETPGTSESATPESSGGSSGGSS. In some embodiments, an RT domain of a gene modifying polypeptide may be located C-terminal to the endonuclease domain. In some

15 embodiments, an RT domain of a gene modifying polypeptide may be located N-terminal to the endonuclease domain.

20

Table 6 Exemplary linker sequences

Amino Acid Sequence	SEQ ID NO
GGS	4101
GGSGGS	4102
GGSGGSGGS	4103
GGSGGSGGSGGS	4104
GGSGGSGGSGGSGGS	4105
GGSGGSGGSGGSGGSGGS	4106
GGGGS	4107
GGGGSGGGGS	4108
GGGGSGGGSGGGGS	4109
GGGGSGGGSGGGSGGGGS	4110

Amino Acid Sequence	SEQ ID NO
GGGGSGGGSGGGSGGGSGGGGS	4111
GGGGSGGGSGGGSGGGSGGGSGGGGS	4112
GGG	4113
GGGG	4114
GGGGG	4115
GGGGGG	4116
GGGGGGG	4117
GGGGGGGG	4118
GSS	4119
GSSGSS	4120
GSSGSSGSS	4121
GSSGSSGSSGSS	4122
GSSGSSGSSGSSGSS	4123
GSSGSSGSSGSSGSSGSS	4124
EAAAK	4125
EAAAKEAAK	4126
EAAAKEAAKEAAK	4127
EAAKEAAKEAAKEAAK	4128
EAAKEAAKEAAKEAAKEAAK	4129
EAAKEAAKEAAKEAAKEAAKEAAK	4130
PAP	4131
PAPAP	4132
PAPAPAP	4133
PAPAPAPAP	4134
PAPAPAPAPAP	4135
PAPAPAPAPAPAP	4136
GGSGGG	4137
GGGGGS	4138
GGSGSS	4139
GSSGGS	4140
GCSEAAK	4141
EAAAKGGS	4142
GGSPAP	4143
PAPGGS	4144
GGGGSS	4145
GSSGGG	4146
GGGEAAK	4147
EAAAKGGG	4148

Amino Acid Sequence	SEQ ID NO
GGGPAP	4149
PAPGGG	4150
GSSEAAK	4151
EAAKGSS	4152
GSSPAP	4153
PAPGSS	4154
EAAKPAP	4155
PAPEAAK	4156
GGSGGGGSS	4157
GGSGSSGGG	4158
GGGGGSGSS	4159
GGGGSSGGS	4160
GSSGGSGGG	4161
GSSGGGGGS	4162
GGSGGGEAAK	4163
GGSEAAKGGG	4164
GGGGGSEAAK	4165
GGGEAAKGGS	4166
EAAKGSGGG	4167
EAAKGSGGGS	4168
GGSGGGPAP	4169
GGSPAPGGG	4170
GGGGGSPAP	4171
GGGPAPGGS	4172
PAPGGSGGG	4173
PAPGGGGGS	4174
GGSGSSEAAK	4175
GGSEAAKGSS	4176
GSSGGSEAAK	4177
GSSEAAKGGG	4178
EAAKGSGGSS	4179
EAAKGSSGGG	4180
GGSGSSPAP	4181
GGSPAPGSS	4182
GSSGGSPAP	4183
GSSPAPGGS	4184
PAPGGGSS	4185
PAPGSSGGG	4186

Amino Acid Sequence	SEQ ID NO
GGSEAAKPAP	4187
GGSPAPEAAK	4188
EAAKGGSPAP	4189
EAAKPAPGGS	4190
PAPGGSEAAK	4191
PAPEAAKGGS	4192
GGGSSEAAK	4193
GCSEAAKGSS	4194
GSSGGSEAAK	4195
GSSEAAKGGG	4196
EAAKGGGGSS	4197
EAAKGGSGGG	4198
GGGGSSPAP	4199
GGGPAPGSS	4200
GSSGGGPAP	4201
GSSPAPGGG	4202
PAPGGGGSS	4203
PAPGSSGGG	4204
GGGEAAKPAP	4205
GGGPAPEAAK	4206
EAAKGGGPAP	4207
EAAKPAPGGG	4208
PAPGGGEAAK	4209
PAPEAAKGGG	4210
GSSEAAKPAP	4211
GSSPAPEAAK	4212
EAAKGGSPAP	4213
EAAKPAPGSS	4214
PAPGSSEAAK	4215
PAPEAAKGSS	4216
AEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKA	4217
GGGSSEAAKGGGS	4218
EAAKGGGSEAAK	4219
SGSETPGTSESATPES	4220
GSAGSAAGSGEF	4221
SGSSGGSSGSETPGTSESATPESGGSSGGSS	4222

In some embodiments, a linker of a gene modifying polypeptide comprises a motif chosen from: (SGGS)_n (SEQ ID NO: 4025), (GGGS)_n (SEQ ID NO: 4026), (GGGGS)_n (SEQ ID NO: 4027), (G)_n, (EAAAK)_n (SEQ ID NO: 4028), (GGS)_n, or (XP)_n.

5 *Gene modifying polypeptide selection by pooled screening*

Candidate gene modifying polypeptides may be screened to evaluate a candidate's gene editing ability. For example, an RNA gene modifying system designed for the targeted editing of a coding sequence in the human genome may be used. In certain embodiments, such a gene modifying system may be used in conjunction with a pooled screening approach.

10 For example, a library of gene modifying polypeptide candidates and a template guide RNA (tgRNA) may be introduced into mammalian cells to test the candidates' gene editing abilities by a pooled screening approach. In specific embodiments, a library of gene modifying polypeptide candidates is introduced into mammalian cells followed by introduction of the tgRNA into the cells.

15 Representative, non-limiting examples of mammalian cells that may be used in screening include HEK293T cells, U2OS cells, HeLa cells, HepG2 cells, Huh7 cells, K562 cells, or iPS cells.

A gene modifying polypeptide candidate may comprise 1) a Cas-nuclease, for example a wild-type Cas nuclease, e.g., a wild-type Cas9 nuclease, a mutant Cas nuclease, e.g., a Cas nickase, for example, a Cas9 nickase such as a Cas9 N863A nickase, or a Cas nuclease selected from **Table**
 20 **3 or Table 4**, 2) a peptide linker, e.g., a sequence from **Table D or Table 6**, that may exhibit varying degrees of length, flexibility, hydrophobicity, and/or secondary structure; and 3) a reverse transcriptase (RT), e.g. an RT domain from **Table D or Table 2**. A gene modifying polypeptide candidate library comprises: a plurality of different gene modifying polypeptide candidates that differ from each other with respect to one, two or all three of the Cas nuclease, peptide linker or
 25 RT domain components, or a plurality of nucleic acid expression vectors that encode such gene modifying polypeptide candidates.

For screening of gene modifying polypeptide candidates, a two-component system may be used that comprises a gene modifying polypeptide component and a tgRNA component. A gene modifying component may comprise, for example, an expression vector, e.g., an expression
 30 plasmid or lentiviral vector, that encodes a gene modifying polypeptide candidate, for example, comprises a human codon-optimized nucleic acid that encodes a gene modifying polypeptide

candidate, e.g., a Cas-linker-RT fusion as described above. In a particular embodiment, a lentiviral cassette is utilized that comprises: (i) a promoter for expression in mammalian cells, e.g., a CMV promoter; (ii) a gene modifying library candidate, e.g. a Cas-linker-RT fusion comprising a Cas nuclease of **Table 3 or Table 4**, a peptide linker of **Table 6**, and an RT of **Table 2**, for example a
5 Cas-linker-RT fusion as in **Table D**; (iii) a self-cleaving polypeptide, e.g., a T2A peptide; (iv) a marker enabling selection in mammalian cells, e.g., a puromycin resistance gene; and (v) a termination signal, e.g., a poly A tail.

The tgRNA component may comprise a tgRNA or expression vector, e.g., an expression plasmid, that produces the tgRNA, for example, utilizes a U6 promoter to drive expression of the
10 tgRNA, wherein the tgRNA is a non-coding RNA sequence that is recognized by Cas and localizes it to the genomic locus of interest, and that also templates reverse transcription of the desired edit into the genome by the RT domain.

To prepare a pool of cells expressing gene modifying polypeptide library candidates, mammalian cells, e.g., HEK293T or U2OS cells, may be transduced with pooled gene modifying
15 polypeptide candidate expression vector preparations, e.g., lentiviral preparations, of the gene modifying candidate polypeptide library. In a particular embodiment, lentiviral plasmids are utilized, and HEK293 Lenti-X cells are seeded in 15 cm plates ($\sim 12 \times 10^6$ cells) prior to lentiviral plasmid transfection. In such an embodiment, lentiviral plasmid transfection may be performed using the Lentiviral Packaging Mix (Biosettia) and transfection of the plasmid DNA for the gene
20 modifying candidate library is performed the following day using Lipofectamine 2000 and Opti-MEM media according to the manufacturer's protocol. In such an embodiment, extracellular DNA may be removed by a full media change the next day and virus-containing media may be harvested 48 hours after. Lentiviral media may be concentrated using Lenti-X Concentrator (TaKaRa Biosciences) and 5 mL lentiviral aliquots may be made and stored at -80°C . Lentiviral titering is
25 performed by enumerating colony forming units post-selection, e.g., post Puromycin selection.

For monitoring gene editing of a target DNA, mammalian cells, e.g., HEK293T or U2OS cells, carrying a target DNA may be utilized. In other embodiments for monitoring gene editing of a target DNA, mammalian cells, e.g., HEK293T or U2OS cells, carrying a target DNA genomic landing pad may be utilized. In particular embodiments, the target DNA genomic landing pad may
30 comprise a gene to be edited for treatment of a disease or disorder of interest. In other particular embodiments, the target DNA is a gene sequence that expresses a protein that exhibits detectable

characteristics that may be monitored to determine whether gene editing has occurred. For example, in certain embodiments, a blue fluorescence protein (BFP)- or green fluorescence protein (GFP)-expressing genomic landing pad is utilized. In certain embodiments, mammalian cells, e.g., HEK293T or U2OS cells, comprising a target DNA, e.g., a target DNA genomic landing pad, are seeded in culture plates at 500x-3000x cells per gene modifying library candidate and transduced at a 0.2-0.3 multiplicity of infection (MOI) to minimize multiple infections per cell. Puromycin (2.5 ug/mL) may be added 48 hours post infection to allow for selection of infected cells. In such an embodiment, cells may be kept under puromycin selection for at least 7 days and then scaled up for tgRNA introduction, e.g., tgRNA electroporation.

To ascertain whether gene editing occurs, mammalian cells containing a target DNA to be edited may be infected with gene modifying polypeptide library candidates then transfected with tgRNA designed for use in editing of the target DNA. Subsequently, the cells may be analyzed to determine whether editing of the target locus has occurred according to the designed outcome, or whether no editing or imperfect editing has occurred, e.g., by using cell sorting and sequence analysis.

In a particular embodiment, to ascertain whether genome editing occurs, BFP- or GFP-expressing mammalian cells, e.g., HEK293T or U2OS cells, may be infected with gene modifying library candidates and then transfected or electroporated with tgRNA plasmid or RNA, e.g., by electroporation of 250,000 cells/well with 200 ng of a tgRNA plasmid designed to convert BFP-to-GFP or GFP-to-BFP, at a cell count ensuring >250x-1000x coverage per library candidate. In such an embodiment, the genome-editing capacity of the various constructs in this assay may be assessed by sorting the cells by Fluorescence-Activated Cell Sorting (FACS) for expression of the color-converted fluorescent protein (FP) at 4-10 days post-electroporation. Cells are sorted and harvested as distinct populations of unedited cells (exhibiting original fluorescence protein signal), edited cells (exhibiting converted fluorescence protein signal), and imperfect edit (exhibiting no fluorescence protein signal) cells. A sample of unsorted cells may also be harvested as the input population to determine candidate enrichment during analysis.

To determine which gene modifying library candidates exhibit genome-editing capacity in an assay, genomic DNA (gDNA) is harvested from the sorted cell populations, and analyzed by sequencing the gene modifying library candidates in each population. Briefly, gene modifying candidates may be amplified from the genome using primers specific to the gene modifying

polypeptide expression vector, e.g., the lentiviral cassette, amplified in a second round of PCR to dilute genomic DNA, and then sequenced, for example, sequenced by a next-generation sequencing platform. After quality control of sequencing reads, reads of at least about 1500 nucleotides and generally no more than about 3200 nucleotides are mapped to the gene modifying
5 polypeptide library sequences and those containing a minimum of about an 80% match to a library sequence are considered to be successfully aligned to a given candidate for purposes of this pooled screen. In order to identify candidates capable of performing gene editing in the assay, e.g., the BFP-to-GFP or GFP-to-BFP edit, the read count of each library candidate in the edited population is compared to its read count in the initial, unsorted population.

10 For purposes of pooled screening, gene modifying candidates with genome-editing capacity are identified based on enrichment in the edited (converted FP) population relative to unsorted (input) cells. In some embodiments, an enrichment of at least 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or at least 100-fold over the input indicates potentially useful gene editing activity, e.g., at least 2-fold enrichment. In some
15 embodiments, the enrichment is converted to a log-value by taking the log base 2 of the enrichment ratio. In some embodiments, a log₂ enrichment score of at least 0, 1, 2, 3, 4, 5, 5.5, 6.0, 6.2, 6.3, 6.4, 6.5, or at least 6.6 indicates potentially useful gene editing activity, e.g., a log₂ enrichment score of at least 1.0. In particular embodiments, enrichment values observed for gene modifying candidates may be compared to enrichment values observed under similar conditions utilizing a
20 reference, e.g., Element ID No: 17380.

In some embodiments, multiple tgRNAs may be used to screen the gene modifying candidate library. In particular embodiments, a plurality of tgRNAs may be utilized to optimize template/Cas-linker-RT fusion pairs, e.g., for gene editing of particular target genes, for example, gene targets for the treatment of disease. In specific embodiments, a pooled approach to screening
25 gene modifying candidates may be performed using a multiplicity of different tgRNAs in an arrayed format.

In some embodiments, multiple types of edits, e.g., insertions, substitutions, and/or deletions of different lengths, may be used to screen the gene modifying candidate library.

In some embodiments, multiple target sequences, e.g., different fluorescent proteins, may
30 be used to screen the gene modifying candidate library. In some embodiments, multiple target sequences, e.g., different fluorescent proteins, may be used to screen the gene modifying candidate

library. In some embodiments, multiple cell types, e.g., HEK293T or U2OS, may be used to screen the gene modifying candidate library. The person of ordinary skill in the art will appreciate that a given candidate may exhibit altered editing capacity or even the gain or loss of any observable or useful activity across different conditions, including tgRNA sequence (e.g., nucleotide
5 modifications, PBS length, RT template length), target sequence, target location, type of edit, location of mutation relative to the first-strand nick of the gene modifying polypeptide, or cell type. Thus, in some embodiments, gene modifying library candidates are screened across multiple parameters, e.g., with at least two distinct tgRNAs in at least two cell types, and gene editing activity is identified by enrichment in any single condition. In other embodiments, a candidate
10 with more robust activity across different tgRNA and cell types is identified by enrichment in at least two conditions, e.g., in all conditions screened. For clarity, candidates found to exhibit little to no enrichment under any given condition are not assumed to be inactive across all conditions and may be screened with different parameters or reconfigured at the polypeptide level, e.g., by swapping, shuffling, or evolving domains (e.g., RT domain), linkers, or other signals (e.g., NLS).

15

Sequences of exemplary Cas9-linker-RT fusions

In some embodiments, a gene modifying polypeptide comprises a linker sequence and an RT sequence. In some embodiments, a gene modifying polypeptide comprises a linker sequence as listed in Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%,
20 97%, 98%, or 99% identity thereto. In some embodiments, a gene modifying polypeptide comprises the amino acid sequence of an RT domain as listed in Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, a gene modifying polypeptide comprises a linker sequence as listed in Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%,
25 98%, or 99% identity thereto; and the amino acid sequence of an RT domain as listed in Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. In some embodiments, a gene modifying polypeptide comprises: (i) a linker sequence as listed in a row of Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto; and (ii) the amino acid sequence of
30 an RT domain as listed in the same row of Table D, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

Localization sequences for gene modifying systems

In certain embodiments, a gene editor system RNA further comprises an intracellular localization sequence, e.g., a nuclear localization sequence (NLS). In some embodiments, a gene
 5 modifying polypeptide comprises an NLS as comprised in SEQ ID NO: 4000 and/or SEQ ID NO: 4001, or an NLS having an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto.

The nuclear localization sequence may be an RNA sequence that promotes the import of the RNA into the nucleus. In certain embodiments the nuclear localization signal is located on
 10 the template RNA. In certain embodiments, the gene modifying polypeptide is encoded on a first RNA, and the template RNA is a second, separate, RNA, and the nuclear localization signal is located on the template RNA and not on an RNA encoding the gene modifying polypeptide. While not wishing to be bound by theory, in some embodiments, the RNA encoding the gene modifying polypeptide is targeted primarily to the cytoplasm to promote its translation, while the
 15 template RNA is targeted primarily to the nucleus to promote insertion into the genome. In some embodiments the nuclear localization signal is at the 3' end, 5' end, or in an internal region of the template RNA. In some embodiments the nuclear localization signal is 3' of the heterologous sequence (e.g., is directly 3' of the heterologous sequence) or is 5' of the heterologous sequence (e.g., is directly 5' of the heterologous sequence). In some embodiments the nuclear localization
 20 signal is placed outside of the 5' UTR or outside of the 3' UTR of the template RNA. In some embodiments the nuclear localization signal is placed between the 5' UTR and the 3' UTR, wherein optionally the nuclear localization signal is not transcribed with the transgene (e.g., the nuclear localization signal is an anti-sense orientation or is downstream of a transcriptional termination signal or polyadenylation signal). In some embodiments the nuclear localization
 25 sequence is situated inside of an intron. In some embodiments a plurality of the same or different nuclear localization signals are in the RNA, e.g., in the template RNA. In some embodiments the nuclear localization signal is less than 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 or 1000 bp in length. Various RNA nuclear localization sequences can be used. For example, Lubelsky and Ulitsky, *Nature* 555 (107-111), 2018 describe RNA
 30 sequences which drive RNA localization into the nucleus. In some embodiments, the nuclear localization signal is a SINE-derived nuclear RNA localization (SIRLOIN) signal. In some

embodiments the nuclear localization signal binds a nuclear-enriched protein. In some embodiments the nuclear localization signal binds the HNRNPK protein. In some embodiments the nuclear localization signal is rich in pyrimidines, e.g., is a C/T rich, C/U rich, C rich, T rich, or U rich region. In some embodiments the nuclear localization signal is derived from a long non-coding RNA. In some embodiments the nuclear localization signal is derived from MALAT1 long non-coding RNA or is the 600 nucleotide M region of MALAT1 (described in Miyagawa et al., *RNA* 18, (738-751), 2012). In some embodiments the nuclear localization signal is derived from BORG long non-coding RNA or is a AGCCC motif (described in Zhang et al., *Molecular and Cellular Biology* 34, 2318-2329 (2014). In some embodiments the nuclear localization sequence is described in Shukla et al., *The EMBO Journal* e98452 (2018). In some embodiments the nuclear localization signal is derived from a retrovirus.

In some embodiments, a polypeptide described herein comprises one or more (e.g., 2, 3, 4, 5) nuclear targeting sequences, for example a nuclear localization sequence (NLS). In some embodiments, the NLS is a bipartite NLS. In some embodiments, an NLS facilitates the import of a protein comprising an NLS into the cell nucleus. In some embodiments, the NLS is fused to the N-terminus of a gene modifying polypeptide as described herein. In some embodiments, the NLS is fused to the C-terminus of the gene modifying polypeptide. In some embodiments, the NLS is fused to the N-terminus or the C-terminus of a Cas domain. In some embodiments, a linker sequence is disposed between the NLS and the neighboring domain of the gene modifying polypeptide.

In some embodiments, an NLS comprises the amino acid sequence MDSLLMNRKFLYQFKNVRWAKGRRETYLC (SEQ ID NO: 4009), PKKRKVEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 4010), RKSGKIAAIWKPRPKPKKKRKV (SEQ ID NO: 4011) KRTADGSEFESPKKKRKV (SEQ ID NO: 4012), KKTELQTTNAENKTKKL (SEQ ID NO: 4013), or KRGINDRNFWRGENGKTR (SEQ ID NO: 4014), KRPAATKKAGQAKKKK (SEQ ID NO: 4015), or a functional fragment or variant thereof. Exemplary NLS sequences are also described in PCT/EP2000/011690, the contents of which are incorporated herein by reference for their disclosure of exemplary nuclear localization sequences. In some embodiments, an NLS comprises an amino acid sequence as disclosed in Table 7. An NLS of this table may be utilized with one or more copies in a polypeptide in one or more locations in a polypeptide, e.g., 1, 2, 3 or more copies of an NLS in

- an N-terminal domain, between peptide domains, in a C-terminal domain, or in a combination of locations, in order to improve subcellular localization to the nucleus. Multiple unique sequences may be used within a single polypeptide. Sequences may be naturally monopartite or bipartite, e.g., having one or two stretches of basic amino acids, or may be used as chimeric bipartite
- 5 sequences. Sequence references correspond to UniProt accession numbers, except where indicated as SeqNLS for sequences mined using a subcellular localization prediction algorithm (Lin et al BMC Bioinform 13:157 (2012), incorporated herein by reference in its entirety).

Table 7 Exemplary nuclear localization signals for use in gene modifying systems

Sequence	Sequence References	SEQ ID No.
AHFKISGEKRPSTDPGKKAKNPKKKKKDP	Q76IQ7	4223
AHRAKKMSKTHA	P21827	4224
ASPEYVNLPIGNG	SeqNLS	4225
CTKRPRW	O88622, Q85W56, Q9QYM2, O02776	4226
DKAKRVSRNKSEKKRR	O15516, Q5RAK8, Q91YB2, Q91YB0, Q8QGQ6, O08785, Q9WVS9, Q6YGZ4	4227
EELRLKEELLKGIYA	Q9QY16, Q9UHL0, Q2TBP1, Q9QY15	4228
EEQLRRRKNSRLNNTG	G5EFF5	4229
EVLKVIRTGKRKKKAWKRMVTKVC	SeqNLS	4230
HHHHHHHHHHHHQPH	Q63934, G3V7L5, Q12837	4231
HKKKHPDASVNFSEFSK	P10103, Q4R844, P12682, B0CM99, A9RA84, Q6YKA4, P09429, P63159, Q08IE6, P63158, Q9YH06, B1MTB0	4232
HKRTKK	Q2R2D5	4233
IINGRKLKLKSSRRSSQTSNNSFTSRRS	SeqNLS	4234
KAEQERRK	Q8LH59	4235
KEKRKRREELFIEQKKRK	SeqNLS	4236
KKGKDEWFSRGKKP	P30999	4237
KKGPSVQKRKKT	Q6ZN17	4238
KKKTVINDLLHYKKEK	SeqNLS, P32354	4239
KKNGGKGKNKPSAKIKK	SeqNLS	4240
KKPKWDDFKKKKK	Q15397, Q8BKS9, Q562C7	4241
KKRKKD	SeqNLS, Q91Z62, Q1A730, Q969P5, Q2KHT6, Q9CPU7	4242
KKRRKRRRK	SeqNLS	4243
KKRRRRARK	Q9UMS6, D4A702, Q91YE8	4244
KKSKRGR	Q9UBS0	4245
KKSRKGS	B4FG96	4246
KKSTALSRELKIMRRR	SeqNLS, P32354	4247
KKSYQDPEIAHSRPRK	Q9U7C9	4248

Sequence	Sequence References	SEQ ID No.
KKTGKNRKLKSKRVKTR	Q9Z301, O54943, Q8K3T2	4249
KKVSIAGQSGKLWRWKR	Q6YUL8	4250
KKYENVVIKRSRKRGRPRK	SeqNLS	4251
KNKKRK	SeqNLS	4252
KPKKKR	SeqNLS	4253
KRAMKDDSHGNSTSPKRRK	Q0E671	4254
KRANSNLVAAYEKAKKK	P23508	4255
KRASEDTTSGSPPKSSAGPKR	Q9BZZ5, Q5R644	4256
KRFKRRWMVRKMKTKK	SeqNLS	4257
KRGLNSSFETSPKKVK	Q8IV63	4258
KRGNSSIGPNDLSKRKQRKK	SeqNLS	4259
KRIHSVLSQSQIDPSKKVKRAK	SeqNLS	4260
KRKGKLNKGSKRKK	O15381	4261
KRRRRRRREKRKR	Q96GM8	4262
KRSNDRTYSPEEEKQRRRA	Q91ZF2	4263
KRTVATNGDASGAHRAKKMSK	SeqNLS	4264
KRVYNKGEDEQEHLPGKKR	SeqNLS	4265
KSGKAPRRRAVSMDNSNK	Q9WVH4, O43524	4266
KVNFLDMSLDIIYKELE	Q9P127	4267
KVQHRIAKTTTRRR	Q9DXE6	4268
LSPLSPL	Q9Y261, P32182, P35583	4269
MDSLLMNRKFLYQFKNVRWAKGRRETYLC	Q9GZX7	4270
MPQNEYIELHRKRYGYRLDYHEKKRKESRE AHERSKAKKMIGLKAKLYHK	SeqNLS	4271
MVQLRPRASR	SeqNLS	4272
NNKLLAKRRKGASPKDDPMDDIK	Q965G5	4273
NYKRPMGTGYPAPKRHEGE	O14497, A2BH40	4274
PDTKRAKLDSETTMVKKK	SeqNLS	4275
PEKRTKI	SeqNLS	4276
PGGRGKKK	Q719N1, Q9UBP0, A2VDN5	4277
PGKMDKGEHRQERRDRPY	Q01844, Q61545	4278
PKKGDKYDKTD	Q45FA5	4279
PKKKSrk	O35914, Q01954	4280
PKKNKPE	Q22663	4281
PKKRAKV	P04295, P89438	4282
PKPKKLKVE	P55263, P55262, P55264, Q64640	4283
PKRGRGR	Q9FY55, Q43386	4284
PKRRLVDDA	P0C797	4285
PKRRRTY	SeqNLS	4286

Sequence	Sequence References	SEQ ID No.
PLFKRR	A8X6H4, Q9TXJ0	4287
PLRKAKR	Q86WB0, Q5R8V9	4288
PPAKRKCIF	Q6AZ28, Q75928, Q8C5D8	4289
PPARRRRL	Q8NAG6	4290
PPKKRKRV	Q3L6L5, P03070, P14999, P03071	4291
PPNKRMMVKH	Q8BN78	4292
PPRIYQLPSAPT	P0C799	4293
PQRSPFPKSSVKR	SeqNLS	4294
PRPRKVPR	P0C799	4295
PRRRVQRKR	SeqNLS, Q5R448, Q5TAQ9	4296
PRRVRLK	Q58DJ0, P56477, Q13568	4297
PSRKPRR	Q62315, Q5F363, Q92833	4298
PSSKKRKV	SeqNLS	4299
PTKKRVK	P07664	4300
QRP GPYDRP	SeqNLS	4301
RGKGGKGLKGAKRHRK	SeqNLS	4302
RKAGGGGGHKTTKRSKDEKVP	B4FG96	4303
RKIKLKRAK	A1L3G9	4304
RKIKRKRAK	B9X187	4305
RKKEAPGPPEELRSRGR	Q35126, P54258, Q5I670, P54259	4306
RKKRKCK	SeqNLS, Q29243, Q62165, Q28685, Q18738, Q9TSZ6, Q14118	4307
RKKRRQRRR	P04326, P69697, P69698, P05907, P20879, P04613, P19553, P0C1J9, P20893, P12506, P04612, Q73370, P0C1K0, P05906, P35965, P04609, P04610, P04614, P04608, P05905	4308
RKKSIPLSIKNLKRKHKKKNKTR	Q9C0C9	4309
RKLVKPKNTKMTKLRTNPY	Q14190	4310
RKRLILSDKGQLDWKK	SeqNLS, Q91Z62, Q1A730, Q2KHT6, Q9CPU7	4311
RKRLKSK	Q13309	4312
RKRRVRDNM	Q8QPH4, Q809M7, A8C8X1, Q2VNC5, Q38SQ0, Q89749, Q6DNQ9, Q809L9, Q0A429, Q20NV3, P16509, P16505, Q6DNQ5, P16506, Q6XT06, P26118, Q2ICQ2, Q2RCG8, Q0A2D0, Q0A2H9, Q9IQ46, Q809M3, Q6J847, Q6J856, B4URE4, A4GCM7, Q0A440, P26120, P16511,	4313
RKRSPDKKEKDLGAGKRRKT	Q7RTP6	4314
RKRTPRVDGQTGENDMNKRRRK	Q94851	4315
RLPVRRRRRR	P04499, P12541, P03269, P48313, P03270	4316
RLRFRKPKSK	P69469	4317
RQQRKR	Q14980	4318
RRDLNSSFETSPKKVK	Q8K3G5	4319
RRDRAKLR	Q9SLB8	4320
RRGDGRRR	Q80WE1, Q5R9B4, Q06787, P35922	4321

Sequence	Sequence References	SEQ ID No.
RRGRKRKAEKQ	Q812D1, Q5XXA9, Q99JF8, Q8MJG1, Q66T72, O75475	4322
RRKKRR	Q0VD86, Q58DS6, Q5R6G2, Q9ERI5, Q6AYK2, Q6NYC1	4323
RRKRSKSEMDSVESKRRR	Q7TT18	4324
RRKRSR	Q99PU7, D3ZHS6, Q92560, A2VDM8	4325
RRPKGKTLQKRKPK	Q6ZN17	4326
RRRGFERFGPDNMGRKRK	Q63014, Q9DBR0	4327
RRRGKNKVAAQNCRK	SeqNLS	4328
RRRKRR	Q5FVH8, Q6MZT1, Q08DH5, Q8BQP9	4329
RRRQKQKGGASRRR	SeqNLS	4330
RRRREGPRARRR	P08313, P10231	4331
RRTIRLKLVDKCDRSCIKKKNRNKCQYCR FHKCLSVGMSHNAIRFGMRPSEKAKLKAE	SeqNLS	4332
RRVPQRKEVSRCKCRK	Q5RJN4, Q32L09, Q8CAK3, Q9NUL5	4333
RVGGRRQAVECIEDLLNEPGQLDLCKRPR P	P03255	4334
RVVKLRAP	P52639, Q8JMN0	4335
RVVRRR	P70278	4336
SKRKTISRKTR	Q5RAY1, Q00443	4337
SYVKTVPNRTRTYIKL	P21935	4338
TGKNEAKKRKIA	P52739, Q8K3J5, Q5RAU9	4339
TLSPASSPSSVSCPVIPASTDESPGSALNI	SeqNLS	4340
VSKKQRTGKKIH	P52739, Q8K3J5, Q5RAU9	4341
SPKKKRKVE		4342
KRTAD GSEFE SPKKKRKVE		4343
PAAKRVKLD		4344
PKKKRKV		4345
MDSLLMNRRKFLYQFKNVRWAKGRRETYLC		4346
SPKKKRKVEAS		4347
MAPKKKRKVGIHRCVP		4348

In some embodiments, the NLS is a bipartite NLS. A bipartite NLS typically comprises two basic amino acid clusters separated by a spacer sequence (which may be, e.g., about 10 amino acids in length). A monopartite NLS typically lacks a spacer. An example of a bipartite NLS is the nucleoplasmin NLS, having the sequence KR[PAATKKAGQA]KKKK (SEQ ID NO: 4015), wherein the spacer is bracketed. Another exemplary bipartite NLS has the sequence PKKKRKVEGADKRTADGSEFESPKKKRKV (SEQ ID NO: 4016). Exemplary NLSs are described in International Application WO2020051561, which is herein incorporated by reference in its entirety, including for its disclosures regarding nuclear localization sequences.

In certain embodiments, a gene editor system polypeptide (e.g., a gene modifying polypeptide as described herein) further comprises an intracellular localization sequence, e.g., a nuclear localization sequence and/or a nucleolar localization sequence. The nuclear localization sequence and/or nucleolar localization sequence may be amino acid sequences that promote the import of the protein into the nucleus and/or nucleolus, where it can promote integration of heterologous sequence into the genome. In certain embodiments, a gene editor system polypeptide (e.g., a gene modifying polypeptide as described herein) further comprises a nucleolar localization sequence. In certain embodiments, the gene modifying polypeptide is encoded on a first RNA, and the template RNA is a second, separate, RNA, and the nucleolar localization signal is encoded on the RNA encoding the gene modifying polypeptide and not on the template RNA. In some embodiments, the nucleolar localization signal is located at the N-terminus, C-terminus, or in an internal region of the polypeptide. In some embodiments, a plurality of the same or different nucleolar localization signals are used. In some embodiments, the nuclear localization signal is less than 5, 10, 25, 50, 75, or 100 amino acids in length. Various polypeptide nucleolar localization signals can be used. For example, Yang et al., *Journal of Biomedical Science* 22, 33 (2015), describe a nuclear localization signal that also functions as a nucleolar localization signal. In some embodiments, the nucleolar localization signal may also be a nuclear localization signal. In some embodiments, the nucleolar localization signal may overlap with a nuclear localization signal. In some embodiments, the nucleolar localization signal may comprise a stretch of basic residues. In some embodiments, the nucleolar localization signal may be rich in arginine and lysine residues. In some embodiments, the nucleolar localization signal may be derived from a protein that is enriched in the nucleolus. In some embodiments, the nucleolar localization signal may be derived from a protein enriched at ribosomal RNA loci. In some embodiments, the nucleolar localization signal may be derived from a protein that binds rRNA. In some embodiments, the nucleolar localization signal may be derived from MSP58. In some embodiments, the nucleolar localization signal may be a monopartite motif. In some embodiments, the nucleolar localization signal may be a bipartite motif. In some embodiments, the nucleolar localization signal may consist of a multiple monopartite or bipartite motifs. In some embodiments, the nucleolar localization signal may consist of a mix of monopartite and bipartite motifs. In some embodiments, the nucleolar localization signal may be a dual bipartite motif. In some embodiments, the nucleolar localization motif may be

a KRASSQALGTIPKRRSSSRFIKRKK (SEQ ID NO: 4017). In some embodiments, the nucleolar localization signal may be derived from nuclear factor- κ B-inducing kinase. In some embodiments, the nucleolar localization signal may be an RKKRKKK motif (SEQ ID NO: 4018) (described in Birbach et al., Journal of Cell Science, 117 (3615-3624), 2004).

5 *Evolved Variants of Gene modifying Polypeptides and Systems*

In some embodiments, the invention provides evolved variants of gene modifying polypeptides as described herein. Evolved variants can, in some embodiments, be produced by mutagenizing a reference gene modifying polypeptide, or one of the fragments or domains comprised therein. In some embodiments, one or more of the domains (e.g., the reverse
10 transcriptase domain) is evolved. One or more of such evolved variant domains can, in some embodiments, be evolved alone or together with other domains. An evolved variant domain or domains may, in some embodiments, be combined with unevolved cognate component(s) or evolved variants of the cognate component(s), e.g., which may have been evolved in either a parallel or serial manner.

15 In some embodiments, the process of mutagenizing a reference gene modifying polypeptide, or fragment or domain thereof, comprises mutagenizing the reference gene modifying polypeptide or fragment or domain thereof. In embodiments, the mutagenesis comprises a continuous evolution method (e.g., PACE) or non-continuous evolution method (e.g., PANCE), e.g., as described herein. In some embodiments, the evolved gene modifying
20 polypeptide, or a fragment or domain thereof, comprises one or more amino acid variations introduced into its amino acid sequence relative to the amino acid sequence of the reference gene modifying polypeptide, or fragment or domain thereof. In embodiments, amino acid sequence variations may include one or more mutated residues (e.g., conservative substitutions, non-conservative substitutions, or a combination thereof) within the amino acid sequence of a
25 reference gene modifying polypeptide, e.g., as a result of a change in the nucleotide sequence encoding the gene modifying polypeptide that results in, e.g., a change in the codon at any particular position in the coding sequence, the deletion of one or more amino acids (e.g., a truncated protein), the insertion of one or more amino acids, or any combination of the foregoing. The evolved variant gene modifying polypeptide may include variants in one or more

components or domains of the gene modifying polypeptide (e.g., variants introduced into a reverse transcriptase domain).

In some aspects, the disclosure provides gene modifying polypeptides, systems, kits, and methods using or comprising an evolved variant of a gene modifying polypeptide, e.g., employs
5 an evolved variant of a gene modifying polypeptide or a gene modifying polypeptide produced or producible by PACE or PANCE. In embodiments, the unevolved reference gene modifying polypeptide is a gene modifying polypeptide as disclosed herein.

The term “phage-assisted continuous evolution (PACE),” as used herein, generally refers to continuous evolution that employs phage as viral vectors. Examples of PACE technology have
10 been described, for example, in International PCT Application No. PCT/US 2009/056194, filed September 8, 2009, published as WO 2010/028347 on March 11, 2010; International PCT Application, PCT/US2011/066747, filed December 22, 2011, published as WO 2012/088381 on June 28, 2012; U.S. Patent No. 9,023,594, issued May 5, 2015; U.S. Patent No. 9,771,574, issued September 26, 2017; U.S. Patent No. 9,394,537, issued July 19, 2016; International PCT
15 Application, PCT/US2015/012022, filed January 20, 2015, published as WO 2015/134121 on September 11, 2015; U.S. Patent No. 10,179,911, issued January 15, 2019; and International PCT Application, PCT/US2016/027795, filed April 15, 2016, published as WO 2016/168631 on October 20, 2016, the entire contents of each of which are incorporated herein by reference.

The term “phage-assisted non-continuous evolution (PANCE),” as used herein, generally
20 refers to non-continuous evolution that employs phage as viral vectors. Examples of PANCE technology have been described, for example, in Suzuki T. et al, Crystal structures reveal an elusive functional domain of pyrrolysyl-tRNA synthetase, Nat Chem Biol. 13(12): 1261-1266 (2017), incorporated herein by reference in its entirety. Briefly, PANCE is a technique for rapid *in vivo* directed evolution using serial flask transfers of evolving selection phage (SP), which
25 contain a gene of interest to be evolved, across fresh host cells (e.g., E. coli cells). Genes inside the host cell may be held constant while genes contained in the SP continuously evolve. Following phage growth, an aliquot of infected cells may be used to transfect a subsequent flask containing host E. coli. This process can be repeated and/or continued until the desired phenotype is evolved, e.g., for as many transfers as desired.

30 Methods of applying PACE and PANCE to gene modifying polypeptides may be readily appreciated by the skilled artisan by reference to, *inter alia*, the foregoing references. Additional

exemplary methods for directing continuous evolution of genome-modifying proteins or systems, e.g., in a population of host cells, e.g., using phage particles, can be applied to generate evolved variants of gene modifying polypeptides, or fragments or subdomains thereof. Non-limiting examples of such methods are described in International PCT Application, PCT/US2009/056194, filed September 8, 2009, published as WO 2010/028347 on March 11, 2010; International PCT Application, PCT/US2011/066747, filed December 22, 2011, published as WO 2012/088381 on June 28, 2012; U.S. Patent No. 9,023,594, issued May 5, 2015; U.S. Patent No. 9,771,574, issued September 26, 2017; U.S. Patent No. 9,394,537, issued July 19, 2016; International PCT Application, PCT/US2015/012022, filed January 20, 2015, published as WO 2015/134121 on September 11, 2015; U.S. Patent No. 10,179,911, issued January 15, 2019; International Application No. PCT/US2019/37216, filed June 14, 2019, International Patent Publication WO 2019/023680, published January 31, 2019, International PCT Application, PCT/US2016/027795, filed April 15, 2016, published as WO 2016/168631 on October 20, 2016, and International Patent Publication No. PCT/US2019/47996, filed August 23, 2019, each of which is incorporated herein by reference in its entirety.

In some non-limiting illustrative embodiments, a method of evolution of a evolved variant gene modifying polypeptide, of a fragment or domain thereof, comprises: (a) contacting a population of host cells with a population of viral vectors comprising the gene of interest (the starting gene modifying polypeptide or fragment or domain thereof), wherein: (1) the host cell is amenable to infection by the viral vector; (2) the host cell expresses viral genes required for the generation of viral particles; (3) the expression of at least one viral gene required for the production of an infectious viral particle is dependent on a function of the gene of interest; and/or (4) the viral vector allows for expression of the protein in the host cell, and can be replicated and packaged into a viral particle by the host cell. In some embodiments, the method comprises (b) contacting the host cells with a mutagen, using host cells with mutations that elevate mutation rate (e.g., either by carrying a mutation plasmid or some genome modification—e.g., proofing-impaired DNA polymerase, SOS genes, such as UmuC, UmuD', and/or RecA, which mutations, if plasmid-bound, may be under control of an inducible promoter), or a combination thereof. In some embodiments, the method comprises (c) incubating the population of host cells under conditions allowing for viral replication and the production of viral particles, wherein host cells are removed from the host cell population, and fresh, uninfected host cells are introduced into the

population of host cells, thus replenishing the population of host cells and creating a flow of host cells. In some embodiments, the cells are incubated under conditions allowing for the gene of interest to acquire a mutation. In some embodiments, the method further comprises (d) isolating a mutated version of the viral vector, encoding an evolved gene product (e.g., an evolved variant
 5 gene modifying polypeptide, or fragment or domain thereof), from the population of host cells.

The skilled artisan will appreciate a variety of features employable within the above-described framework. For example, in some embodiments, the viral vector or the phage is a filamentous phage, for example, an M13 phage, e.g., an M13 selection phage. In certain embodiments, the gene required for the production of infectious viral particles is the M13 gene
 10 III (gIII). In embodiments, the phage may lack a functional gIII, but otherwise comprise gI, gII, gIV, gV, gVI, gVII, gVIII, gIX, and a gX. In some embodiments, the generation of infectious VSV particles involves the envelope protein VSV-G. Various embodiments can use different retroviral vectors, for example, Murine Leukemia Virus vectors, or Lentiviral vectors. In embodiments, the retroviral vectors can efficiently be packaged with VSV-G envelope protein,
 15 e.g., as a substitute for the native envelope protein of the virus.

In some embodiments, host cells are incubated according to a suitable number of viral life cycles, e.g., at least 10, at least 20, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1000, at least 1250, at least 1500, at least 1750, at least 2000, at least 2500, at least 3000, at
 20 least 4000, at least 5000, at least 7500, at least 10000, or more consecutive viral life cycles, which in on illustrative and non-limiting examples of M13 phage is 10-20 minutes per virus life cycle. Similarly, conditions can be modulated to adjust the time a host cell remains in a population of host cells, e.g., about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25,
 25 about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 70, about 80, about 90, about 100, about 120, about 150, or about 180 minutes. Host cell populations can be controlled in part by density of the host cells, or, in some embodiments, the host cell density in an inflow, e.g., 10^3 cells/ml, about 10^4 cells/ml, about 10^5 cells/ml, about $5 \cdot 10^5$ cells/ml, about 10^6 cells/ml, about $5 \cdot 10^6$ cells/ml, about 10^7 cells/ml, about $5 \cdot 10^7$ cells/ml, about 10^8 cells/ml,
 30 about $5 \cdot 10^8$ cells/ml, about 10^9 cells/ml, about $5 \cdot 10^9$ cells/ml, about 10^{10} cells/ml, or about $5 \cdot 10^{10}$ cells/ml.

Inteins

In some embodiments, as described in more detail below, an intein-N (intN) domain may be fused to the N-terminal portion of a first domain of a gene modifying polypeptide described herein, and an intein-C (intC) domain may be fused to the C-terminal portion of a second domain of a gene modifying polypeptide described herein for the joining of the N-terminal portion to the C-terminal portion, thereby joining the first and second domains. In some embodiments, the first and second domains are each independently chosen from a DNA binding domain, an RNA binding domain, an RT domain, and an endonuclease domain.

Inteins can occur as self-splicing protein intron (e.g., peptide), e.g., which ligates flanking N-terminal and C-terminal exteins (e.g., fragments to be joined). An intein may, in some instances, comprise a fragment of a protein that is able to excise itself and join the remaining fragments (the exteins) with a peptide bond in a process known as protein splicing. Inteins are also referred to as “protein introns.” The process of an intein excising itself and joining the remaining portions of the protein is herein termed “protein splicing” or “intein-mediated protein splicing.”

In some embodiments, an intein of a precursor protein (an intein containing protein prior to intein-mediated protein splicing) comes from two genes. Such intein is referred to herein as a split intein (e.g., split intein-N and split intein-C). Accordingly, an intein-based approach may be used to join a first polypeptide sequence and a second polypeptide sequence together. For example, in cyanobacteria, DnaE, the catalytic subunit a of DNA polymerase III, is encoded by two separate genes, dnaE-n and dnaE-c. An intein-N domain, such as that encoded by the dnaE-n gene, when situated as part of a first polypeptide sequence, may join the first polypeptide sequence with a second polypeptide sequence, wherein the second polypeptide sequence comprises an intein-C domain, such as that encoded by the dnaE-c gene. Accordingly, in some embodiments, a protein can be made by providing nucleic acid encoding the first and second polypeptide sequences (e.g., wherein a first nucleic acid molecule encodes the first polypeptide sequence and a second nucleic acid molecule encodes the second polypeptide sequence), and the nucleic acid is introduced into the cell under conditions that allow for production of the first and second polypeptide sequences, and for joining of the first to the second polypeptide sequence via an intein-based mechanism.

Use of inteins for joining heterologous protein fragments is described, for example, in Wood et al., J. Biol. Chem. 289(21): 14512-9 (2014) (incorporated herein by reference in its entirety). For example, when fused to separate protein fragments, the inteins IntN and IntC may recognize each other, splice themselves out, and/or simultaneously ligate the flanking N- and C-terminal exteins of the protein fragments to which they were fused, thereby reconstituting a full-length protein from the two protein fragments.

In some embodiments, a synthetic intein based on the dnaE intein, the Cfa-N (e.g., split intein-N) and Cfa-C (e.g., split intein-C) intein pair, is used. Examples of such inteins have been described, e.g., in Stevens et al., J Am Chem Soc. 2016 Feb. 24; 138(7):2162-5 (incorporated herein by reference in its entirety). Non-limiting examples of intein pairs that may be used in accordance with the present disclosure include: Cfa DnaE intein, Ssp GyrB intein, Ssp DnaX intein, Ter DnaE3 intein, Ter ThyX intein, Rma DnaB intein and Cne Prp8 intein (e.g., as described in U.S. Pat. No. 8,394,604, incorporated herein by reference.

In some embodiments involving a split Cas9, an intein-N domain and an intein-C domain may be fused to the N-terminal portion of the split Cas9 and the C-terminal portion of a split Cas9, respectively, for the joining of the N-terminal portion of the split Cas9 and the C-terminal portion of the split Cas9. For example, in some embodiments, an intein-N is fused to the C-terminus of the N-terminal portion of the split Cas9, i.e., to form a structure of N— [N-terminal portion of the split Cas9]-[intein-N]~C. In some embodiments, an intein-C is fused to the N-terminus of the C-terminal portion of the split Cas9, i.e., to form a structure of N-[intein-C]~[C-terminal portion of the split Cas9]-C. The mechanism of intein-mediated protein splicing for joining the proteins the inteins are fused to (e.g., split Cas9) is described in Shah et al., Chem Sci. 2014; 5(1):446-461, incorporated herein by reference. Methods for designing and using inteins are known in the art and described, for example by WO2020051561, W02014004336, WO2017132580, US20150344549, and US20180127780, each of which is incorporated herein by reference in their entirety.

In some embodiments, a split refers to a division into two or more fragments. In some embodiments, a split Cas9 protein or split Cas9 comprises a Cas9 protein that is provided as an N-terminal fragment and a C-terminal fragment encoded by two separate nucleotide sequences. The polypeptides corresponding to the N-terminal portion and the C-terminal portion of the Cas9 protein may be spliced to form a reconstituted Cas9 protein. In embodiments, the Cas9 protein is

divided into two fragments within a disordered region of the protein, e.g., as described in Nishimasu et al., Cell, Volume 156, Issue 5, pp. 935-949, 2014, or as described in Jiang et al. (2016) Science 351: 867-871 and PDB file: 5F9R (each of which is incorporated herein by reference in its entirety). A disordered region may be determined by one or more protein
5 structure determination techniques known in the art, including, without limitation, X-ray crystallography, NMR spectroscopy, electron microscopy (e.g., cryoEM), and/or *in silico* protein modeling. In some embodiments, the protein is divided into two fragments at any C, T, A, or S, e.g., within a region of SpCas9 between amino acids A292- G364, F445-K483, or E565-T637, or at corresponding positions in any other Cas9, Cas9 variant (e.g., nCas9, dCas9), or other
10 napDNAbp. In some embodiments, protein is divided into two fragments at SpCas9 T310, T313, A456, S469, or C574. In some embodiments, the process of dividing the protein into two fragments is referred to as splitting the protein.

In some embodiments, a protein fragment ranges from about 2-1000 amino acids (e.g., between 2-10, 10-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800,
15 800-900, or 900-1000 amino acids) in length. In some embodiments, a protein fragment ranges from about 5-500 amino acids (e.g., between 5-10, 10-50, 50-100, 100-200, 200-300, 300-400, or 400-500 amino acids) in length. In some embodiments, a protein fragment ranges from about 20-200 amino acids (e.g., between 20-30, 30-40, 40-50, 50-100, or 100-200 amino acids) in length.

In some embodiments, a portion or fragment of a gene modifying polypeptide is fused to
20 an intein. The nuclease can be fused to the N-terminus or the C-terminus of the intein. In some embodiments, a portion or fragment of a fusion protein is fused to an intein and fused to an AAV capsid protein. The intein, nuclease and capsid protein can be fused together in any arrangement (e.g., nuclease-intein-capsid, intein-nuclease-capsid, capsid-intein-nuclease, etc.). In some
25 C-terminus of the intein is fused to the N-terminus of an AAV capsid protein.

In some embodiments, an endonuclease domain (e.g., a nickase Cas9 domain) is fused to intein-N and a polypeptide comprising an RT domain is fused to an intein-C.

Exemplary nucleotide and amino acid sequences of intein-N domains and compatible intein-C domains are provided below:

30

DnaE Intein-N DNA:

TGCCTGTCATACGAAACCGAGATACTGACAGTAGAATATGGCCTTCTGCCAATCGGG
AAGATTGTGGAGAAACGGATAGAATGCACAGTTTACTCTGTGCGATAACAATGGTAA
CATTTATACTCAGCCAGTTGCCAGTGGCACGACCGGGGAGAGCAGGAAGTATTCG
5 AATACTGTCTGGAGGATGGAAGTCTCATTAGGGGCCACTAAGGACCACAAATTTATG
ACAGTCGATGGCCAGATGCTGCCTATAGACGAAATCTTTGAGCGAGAGTTGGACCTC
ATGCGAGTTGACAACCTTCCTAAT (SEQ ID NO: 4029)

DnaE Intein-N Protein:

10 CLSYETEILTVEYGLLPIGKIVEKRIECTVYSVDNNGNIYTQPV AQWHDRGEQEVFEYCL
EDGSLIRATKDHKFMFTVDGQMLPIDEIFERELDLMRVDNLPN (SEQ ID NO: 4030)

DnaE Intein-C DNA:

15 ATGATCAAGATAGCTACAAGGAAGTATCTTGGCAAACAAAACGTTTATGATATTGG
AGTCGAAAGAGATCACAACCTTTGCTCTGAAGAACGGATTCATAGCTTCTAAT (SEQ
ID NO: 4031)

DnaE Intein-C Protein:

20 MIKIATRKYLGKQNVYDIGVERDHN FALKNGFIASN (SEQ ID NO: 4032)

Cfa-N DNA:

TGCCTGTCTTATGATACCGAGATACTTACCGTTGAATATGGCTTCTTGCCTATTGGAA
AGATTGTGCGAAGAGAGAATTGAATGCACAGTATATACTGTAGACAAGAATGGTTTC
GTTTACACACAGCCCATTGCTCAATGGCACAATCGCGGCGAACAAGAAGTATTTGA
25 GTACTGTCTCGAGGATGGAAGCATCATACGAGCAACTAAAGATCATAAATTCATGA
CCACTGACGGGCAGATGTTGCCAATAGATGAGATATTCGAGCGGGGCTTGATCTC
AAACAAGTGGATGGATTG CCA (SEQ ID NO: 4033)

Cfa-N Protein:

30 CLSYDTEILTVEYGFLLPIGKIVEERIECTVYTVDKNGFVYTQPIAQWHNRGEQEVFEYCL
EDGSIIRATKDHKFMFTTDGQMLPIDEIFERGLDLKQVDGLP (SEQ ID NO: 4034)

Cfa-C DNA:

35 ATGAAGAGGACTGCCGATGGATCAGAGTTTGAATCTCCCAAGAAGAAGAGGAAAGT
AAAGATAATATCTCGAAAAAGTCTTGGTACCCAAAATGTCTATGATATTGGAGTGG
GAAAGATCACAACCTTCCTTCTCAAGAACGGTCTCGTAGCCAGCAAC (SEQ ID NO:
4035)

Cfa-C Protein:

40 MKRTADGSEFESPKKKRKVKIISRKSLGTQNVYDIGVEKDHNFLLKNGLVASN (SEQ ID
NO: 4036)

Additional domains

The gene modifying polypeptide can bind a target DNA sequence and template nucleic acid (e.g., template RNA), nick the target site, and write (e.g., reverse transcribe) the template into DNA, resulting in a modification of the target site. In some embodiments, additional domains may be added to the polypeptide to enhance the efficiency of the process. In some
 5 embodiments, the gene modifying polypeptide may contain an additional DNA ligation domain to join reverse transcribed DNA to the DNA of the target site. In some embodiments, the polypeptide may comprise a heterologous RNA-binding domain. In some embodiments, the polypeptide may comprise a domain having 5' to 3' exonuclease activity (e.g., wherein the 5' to 3' exonuclease activity increases repair of the alteration of the target site, e.g., in favor of
 10 alteration over the original genomic sequence). In some embodiments, the polypeptide may comprise a domain having 3' to 5' exonuclease activity, e.g., proof-reading activity. In some embodiments, the writing domain, e.g., RT domain, has 3' to 5' exonuclease activity, e.g., proof-reading activity.

15 *Template nucleic acids*

The gene modifying systems described herein can modify a host target DNA site using a template nucleic acid sequence. In some embodiments, the gene modifying systems described herein transcribe an RNA sequence template into host target DNA sites by target-primed reverse transcription (TPRT). By modifying DNA sequence(s) via reverse transcription of the RNA
 20 sequence template directly into the host genome, the gene modifying system can insert an object sequence into a target genome without the need for exogenous DNA sequences to be introduced into the host cell (unlike, for example, CRISPR systems), as well as eliminate an exogenous DNA insertion step. The gene modifying system can also delete a sequence from the target genome or introduce a substitution using an object sequence. Therefore, the gene modifying
 25 system provides a platform for the use of customized RNA sequence templates containing object sequences, e.g., sequences comprising heterologous gene coding and/or function information.

In some embodiments, the template nucleic acid comprises one or more sequence (e.g., 2 sequences) that binds the gene modifying polypeptide.

In some embodiments, the template nucleic acid comprises a hybrid having both
 30 ribonucleotide and deoxyribonucleotide residues in the same strand.

In some embodiments a system or method described herein comprises a single template nucleic acid (e.g., template RNA). In some embodiments a system or method described herein comprises a plurality of template nucleic acids (e.g., template RNAs). For example, a system described herein comprises a first RNA comprising (e.g., from 5' to 3') a sequence that binds the gene modifying polypeptide (e.g., the DNA-binding domain and/or the endonuclease domain, e.g., a gRNA) and a sequence that binds a target site (e.g., a second strand of a site in a target genome), and a second RNA (e.g., a template RNA) comprising (e.g., from 5' to 3') optionally a sequence that binds the gene modifying polypeptide (e.g., that specifically binds the RT domain), a heterologous object sequence, and a PBS sequence. In some embodiments, when the system comprises a plurality of nucleic acids, each nucleic acid comprises a conjugating domain. In some embodiments, a conjugating domain enables association of nucleic acid molecules, e.g., by hybridization of complementary sequences. For example, in some embodiments a first RNA comprises a first conjugating domain and a second RNA comprises a second conjugating domain, and the first and second conjugating domains are capable of hybridizing to one another, e.g., under stringent conditions. In some embodiments, the stringent conditions for hybridization include hybridization in 4x sodium chloride/sodium citrate (SSC), at about 65 C, followed by a wash in 1xSSC, at about 65 C.

In some embodiments, the template nucleic acid comprises RNA. In some embodiments, the template nucleic acid comprises DNA (e.g., single stranded or double stranded DNA). In some embodiments, the template nucleic acid comprises a hybrid having both ribonucleotide and deoxyribonucleotide residues in the same strand.

In some embodiments, the template nucleic acid comprises one or more (e.g., 2) homology domains that have homology to the target sequence. In some embodiments, the homology domains are about 10-20, 20-50, or 50-100 nucleotides in length.

In some embodiments, a template RNA can comprise a gRNA sequence, e.g., to direct the gene modifying polypeptide to a target site of interest. In some embodiments, a template RNA comprises (e.g., from 5' to 3') (i) optionally a gRNA spacer that binds a target site (e.g., a second strand of a site in a target genome), (ii) optionally a gRNA scaffold that binds a polypeptide described herein (e.g., a gene modifying polypeptide or a Cas polypeptide), (iii) a heterologous object sequence comprising a mutation region (optionally the heterologous object

sequence comprises, from 5' to 3', a first homology region, a mutation region, and a second homology region), and (iv) a primer binding site (PBS) sequence comprising a 3' target homology domain.

The template nucleic acid (e.g., template RNA) component of a genome editing system described herein typically is able to bind the gene modifying polypeptide of the system. In some embodiments the template nucleic acid (e.g., template RNA) has a 3' region that is capable of binding a gene modifying polypeptide. The binding region, e.g., 3' region, may be a structured RNA region, e.g., having at least 1, 2 or 3 hairpin loops, capable of binding the gene modifying polypeptide of the system. The binding region may associate the template nucleic acid (e.g., template RNA) with any of the polypeptide modules. In some embodiments, the binding region of the template nucleic acid (e.g., template RNA) may associate with an RNA-binding domain in the polypeptide. In some embodiments, the binding region of the template nucleic acid (e.g., template RNA) may associate with the reverse transcription domain of the gene modifying polypeptide (e.g., specifically bind to the RT domain). In some embodiments, the template nucleic acid (e.g., template RNA) may associate with the DNA binding domain of the polypeptide, e.g., a gRNA associating with a Cas9-derived DNA binding domain. In some embodiments, the binding region may also provide DNA target recognition, e.g., a gRNA hybridizing to the target DNA sequence and binding the polypeptide, e.g., a Cas9 domain. In some embodiments, the template nucleic acid (e.g., template RNA) may associate with multiple components of the polypeptide, e.g., DNA binding domain and reverse transcription domain.

In some embodiments the template RNA has a poly-A tail at the 3' end. In some embodiments the template RNA does not have a poly-A tail at the 3' end.

In some embodiments, a template RNA may be customized to correct a given mutation in the genomic DNA of a target cell (e.g., *ex vivo* or *in vivo*, e.g., in a target tissue or organ, e.g., in a subject). For example, the mutation may be a disease-associated mutation relative to the wild-type sequence. Without wishing to be bound by theory, any given target site and edit will have a large number of possible template RNA molecules for use in a gene modifying system that will result in a range of editing efficiencies and fidelities. To partially reduce this screening burden, sets of empirical parameters help ensure optimal initial *in silico* designs of template RNAs or portions thereof. As a non-limiting illustrative example, for a selected mutation, the following design parameters may be employed. In some embodiments, design is initiated by acquiring

approximately 500 bp (e.g., up to 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or 700 bp, and optionally at least 20, 30, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, or 650 bp) flanking sequence on either side of the mutation to serve as the target region. In some embodiments, a template nucleic acid comprises a gRNA. In some

5 embodiments, a gRNA comprises a sequence (e.g., a CRISPR spacer) that binds a target site. In some embodiments, the sequence (e.g., a CRISPR spacer) that binds a target site for use in targeting a template nucleic acid to a target region is selected by considering the particular gene modifying polypeptide (e.g., endonuclease domain or writing domain, e.g., comprising a CRISPR/Cas domain) being used (e.g., for Cas9, a protospacer-adjacent motif (PAM) of NGG

10 immediately 3' of a 20 nucleotide gRNA binding region). In some embodiments, the CRISPR spacer is selected by ranking first by whether the PAM will be disrupted by the gene modifying system induced edit. In some embodiments, disruption of the PAM may increase edit efficiency. In some embodiments, the PAM can be disrupted by also introducing (e.g., as part of or in addition to another modification to a target site in genomic DNA) a silent mutation (e.g., a

15 mutation that does not alter an amino acid residue encoded by the target nucleic acid sequence, if any) in the target site during gene modification. In some embodiments, the CRISPR spacer is selected by ranking sequences by the proximity of their corresponding genomic site to the desired edit location. In some embodiments, the gRNA comprises a gRNA scaffold. In some embodiments, the gRNA scaffold used may be a standard scaffold (e.g., for Cas9, 5'-

20 GTTTGTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGC-3'), or may contain one or more nucleotide substitutions. In some embodiments, the heterologous object sequence has at least 90% identity, e.g., at least 90%, 95%, 98%, 99%, or 100% identity, or comprises no more than 1, 2, 3, 4, or 5 positions of non-identity to the target site 3' of the first strand nick (e.g., immediately 3' of the first strand

25 nick or up to 1, 2, 3, 4, or 5 nucleotides 3' of the first strand nick), with the exception of any insertion, substitution, or deletion that may be written into the target site by the gene modifying. In some embodiments, the 3' target homology domain contains at least 90% identity, e.g., at least 90%, 95%, 98%, 99%, or 100% identity, or comprises no more than 1, 2, 3, 4, or 5 positions of non-identity to the target site 5' of the first strand nick (e.g., immediately 5' of the first strand

30 nick or up to 1, 2, 3, 4, or 5 nucleotides 3' of the first strand nick).

In some embodiments, the template nucleic acid is a template RNA. In some embodiments, the template RNA comprises one or more modified nucleotides. For example, in some embodiments, the template RNA comprises one or more deoxyribonucleotides. In some embodiments, regions of the template RNA are replaced by DNA nucleotides, e.g., to enhance stability of the molecule. For example, the 3' end of the template may comprise DNA nucleotides, while the rest of the template comprises RNA nucleotides that can be reverse transcribed. For instance, in some embodiments, the heterologous object sequence is primarily or wholly made up of RNA nucleotides (e.g., at least 90%, 95%, 98%, or 99% RNA nucleotides). In some embodiments, the PBS sequence is primarily or wholly made up of DNA nucleotides (e.g., at least 90%, 95%, 98%, or 99% DNA nucleotides). In other embodiments, the heterologous object sequence for writing into the genome may comprise DNA nucleotides. In some embodiments, the DNA nucleotides in the template are copied into the genome by a domain capable of DNA-dependent DNA polymerase activity. In some embodiments, the DNA-dependent DNA polymerase activity is provided by a DNA polymerase domain in the polypeptide. In some embodiments, the DNA-dependent DNA polymerase activity is provided by a reverse transcriptase domain that is also capable of DNA-dependent DNA polymerization, e.g., second strand synthesis. In some embodiments, the template molecule is composed of only DNA nucleotides. In some embodiments, the template nucleic acid comprises a hybrid having both ribonucleotide and deoxyribonucleotide residues in the same strand.

In some embodiments, a system described herein comprises two nucleic acids which together comprise the sequences of a template RNA described herein. In some embodiments, the two nucleic acids are associated with each other non-covalently, e.g., directly associated with each other (e.g., via base pairing), or indirectly associated as part of a complex comprising one or more additional molecule.

A template RNA described herein may comprise, from 5' to 3': (1) a gRNA spacer; (2) a gRNA scaffold; (3) heterologous object sequence (4) a primer binding site (PBS) sequence. Each of these components is now described in more detail.

gRNA spacer and gRNA scaffold

A template RNA described herein may comprise a gRNA spacer that directs the gene modifying system to a target nucleic acid, and a gRNA scaffold that promotes association of the

template RNA with the Cas domain of the gene modifying polypeptide. The systems described herein can also comprise a gRNA that is not part of a template nucleic acid. For example, a gRNA that comprises a gRNA spacer and gRNA scaffold, but not a heterologous object sequence or a PBS sequence, can be used, e.g., to induce second strand nicking, e.g., as described in the section herein entitled “Second Strand Nicking”.

In some embodiments, the gRNA is a short synthetic RNA composed of a scaffold sequence that participates in CRISPR-associated protein binding and a user-defined ~20 nucleotide targeting sequence for a genomic target. The structure of a complete gRNA was described by Nishimasu et al. Cell 156, P935-949 (2014). The gRNA (also referred to as sgRNA for single-guide RNA) consists of crRNA- and tracrRNA-derived sequences connected by an artificial tetraloop. The crRNA sequence can be divided into guide (20 nt) and repeat (12 nt) regions, whereas the tracrRNA sequence can be divided into anti-repeat (14 nt) and three tracrRNA stem loops (Nishimasu et al. Cell 156, P935-949 (2014)). In practice, guide RNA sequences are generally designed to have a length of between 17 – 24 nucleotides (e.g., 19, 20, or 21 nucleotides) and be complementary to a targeted nucleic acid sequence. Custom gRNA generators and algorithms are available commercially for use in the design of effective guide RNAs. In some embodiments, the gRNA comprises two RNA components from the native CRISPR system, e.g. crRNA and tracrRNA. As is well known in the art, the gRNA may also comprise a chimeric, single guide RNA (sgRNA) containing sequence from both a tracrRNA (for binding the nuclease) and at least one crRNA (to guide the nuclease to the sequence targeted for editing/binding). Chemically modified sgRNAs have also been demonstrated to be effective for use with CRISPR-associated proteins; see, for example, Hendel et al. (2015) Nature Biotechnol., 985 – 991. In some embodiments, a gRNA spacer comprises a nucleic acid sequence that is complementary to a DNA sequence associated with a target gene.

In some embodiments, the region of the template nucleic acid, e.g., template RNA, comprising the gRNA adopts an underwound ribbon-like structure of gRNA bound to target DNA (e.g., as described in Mulepati et al. Science 19 Sep 2014:Vol. 345, Issue 6203, pp. 1479-1484). Without wishing to be bound by theory, this non-canonical structure is thought to be facilitated by rotation of every sixth nucleotide out of the RNA-DNA hybrid. Thus, in some embodiments, the region of the template nucleic acid, e.g., template RNA, comprising the gRNA may tolerate increased mismatching with the target site at some interval, e.g., every sixth base. In

some embodiments, the region of the template nucleic acid, e.g., template RNA, comprising the gRNA comprising homology to the target site may possess wobble positions at a regular interval, e.g., every sixth base, that do not need to base pair with the target site.

In some embodiments, the template nucleic acid (e.g., template RNA) has at least 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 bases of at least 80%, 85%, 90%, 95%, 99%, or 100% homology to the target site, e.g., at the 5' end, e.g., comprising a gRNA spacer sequence of length appropriate to the Cas9 domain of the gene modifying polypeptide (Table 3).

In some embodiments, a Cas9 derivative with enhanced activity may be used in the gene modification polypeptide. In some embodiments, a Cas9 derivative may comprise mutations that improve activity of the HNH endonuclease domain, e.g., SpyCas9 R221K, N394K, or mutations that improve R-loop formation, e.g., SpyCas9 L1245V, or comprise a combination of such mutations, e.g., SpyCas9 R221K/N394K, SpyCas9 N394K/L1245V, SpyCas9 R221K/L1245V, or SpyCas9 R221K/N394K/L1245V (see, e.g., Spencer and Zhang Sci Rep 7:16836 (2017), the Cas9 derivatives and comprising mutations of which are incorporated herein by reference). In some embodiments, a Cas9 derivative may comprise one or more types of mutations described herein, e.g., PAM-modifying mutations, protein stabilizing mutations, activity enhancing mutations, and/or mutations partially or fully inactivating one or two endonuclease domains relative to the parental enzyme (e.g., one or more mutations to abolish endonuclease activity towards one or both strands of a target DNA, e.g., a nickase or catalytically dead enzyme). In some embodiments, a Cas9 enzyme used in a system described herein may comprise mutations that confer nickase activity toward the enzyme (e.g., SpyCas9 N863A or H840A) in addition to mutations improving catalytic efficiency (e.g., SpyCas9 R221K, N394K, and/or L1245V). In some embodiments, a Cas9 enzyme used in a system described herein is a SpyCas9 enzyme or derivative that further comprises an N863A mutation to confer nickase activity in addition to R221K and N394K mutations to improve catalytic efficiency.

Table 8 provides parameters to define components for designing gRNA and/or Template RNAs to apply Cas variants listed in Table 3 for gene modifying. The cut site indicates the validated or predicted protospacer adjacent motif (PAM) requirements, validated or predicted location of cut site (relative to the most upstream base of the PAM site). The gRNA for a given enzyme can be assembled by concatenating the crRNA, Tetraloop, and tracrRNA sequences, and further adding a 5' spacer of a length within Spacer (min) and Spacer (max) that matches a

protospacer at a target site. Further, the predicted location of the ssDNA nick at the target is important for designing a PBS sequence of a Template RNA that can anneal to the sequence immediately 5' of the nick in order to initiate target primed reverse transcription. In some embodiments, a gRNA scaffold described herein comprises a nucleic acid sequence comprising, in the 5' to 3' direction, a crRNA of Table 8, a tetraloop from the same row of Table 8, and a tracrRNA from the same row of Table 8, or a sequence having at least 70%, 80%, 85%, 90%, 95%, or 99% identity thereto. In some embodiments, the gRNA or template RNA comprising the scaffold further comprises a gRNA spacer having a length within the Spacer (min) and Spacer (max) indicated in the same row of Table 8. In some embodiments, the gRNA or template RNA having a sequence according to Table 8 is comprised by a system that further comprises a gene modifying polypeptide, wherein the gene modifying polypeptide comprises a Cas domain described in the same row of Table 8.

Table 8 Parameters to define components for designing gRNA and/or Template RNAs to apply Cas variants listed in Table 3 in gene modifying systems

Variant	PAM(s)	Cut	Tie r	Spac er (min)	Spac er (max)	crRNA	Tetralo op	tracrRNA
Nme2Cas9	NNNN CC	-3	1	22	24	GTTGTA GCTCCC TTTCTC ATTTTCG	GAAA	CGAAATGAGAACCGTTGCTAC AATAAGGCCGCTCTGAAAAGAT GTGCCGCAACGCTCTGCCCTT AAAGCTTCTGCTTTAAGGGGC ATCGTTTA
PpnCas9	NNNN RTT		1	21	24	GTTGTA GCTCCC TTTTTC ATTTTCG C	GAAA	GCGAAATGAAAAACGTTGTTA CAATAAGAGATGAATTTCTCG CAAAGCTCTGCCTCTTGAAATT TCGTTTCAAGAGGCATC
SauCas9	NNGRR ;NNGR RT	-3	1	21	23	GTTTTA GTACTC TG	GAAA	CAGAATCTACTAAAACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
SauCas9-KKH	NNNRR ;NNNR RT	-3	1	21	21	GTTTTA GTACTC TG	GAAA	CAGAATCTACTAAAACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA

SauriCas9	NNGG	-3	1	21	21	GTTTTA GTACTC TG	GAAA	CAGAATCTACTAAAACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
SauriCas9- KKH	NNRG	-3	1	21	21	GTTTTA GTACTC TG	GAAA	CAGAATCTACTAAAACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
ScaCas9- Sc++	NNG	-3	1	20	20	GTTTTA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9	NGG	-3	1	20	20	GTTTTA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9-NG	NG (NGG= NGA=N GT>NG C)	-3	1	20	20	GTTTTA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9- SpRY	NRN>N YN	-3	1	20	20	GTTTTA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
St1Cas9	NNAGA AW>N NAGGA W=NN GGAA W	-3	1	20	20	GTCTTT GTACTC TG	GTAC	CAGAAGCTACAAAGATAAGGC TTCATGCCGAAATCAACACCCT GTCATTTTATGGCAGGGTGTT TT
BlatCas9	NNNN CNA> NNNN CNDD> NNNN C	-3	1	19	23	GCTATA GTTCC TACT	GAAA	GGTAAGTTGCTATAGTAAGGG CAACAGACCCGAGGCGTTGG GGATCGCCTAGCCCGTGTTTA CGGGCTCTCCCATATTCAAAA TAATGACAGACGAGCACCTTG GAGCATTTATCTCCGAGGTGC T
cCas9-v16	NNVAC T;NNV ATGM; NNVAT T;NNV GCT;N	-3	2	21	21	GTCTTA GTACTC TG	GAAA	CAGAATCTACTAAGACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA

	NVGTG ;NNVG TT							
cCas9-v17	NNVRR N	-3	2	21	21	GTCTTA GTACTC TG	GAAA	CAGAATCTACTAAGACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
cCas9-v21	NNVAC T;NNV ATGM; NNVAT T;NNV GCT;N NVGTG ;NNVG TT	-3	2	21	21	GTCTTA GTACTC TG	GAAA	CAGAATCTACTAAGACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
cCas9-v42	NNVRR N	-3	2	21	21	GTCTTA GTACTC TG	GAAA	CAGAATCTACTAAGACAAGGC AAAATGCCGTGTTTATCTCGTC AACTTGTTGGCGAGA
CdiCas9	NNRHH HY;NN RAAAY		2	22	22	ACTGGG GTTGAG	GAAA	CTGAACCTCAGTAAGCATTGG CTCGTTTCCAATGTTGATTGCT CCGCCGGTGCTCCTTATTTTA AGGGCGCCGGC
CjeCas9	NNNN RYAC	-3	2	21	23	GTTTTA GTCCTT	GAAA	AGGGACTAAAATAAGAGTTT GCGGGACTCTGCGGGGTTACA ATCCCCATAAACCGC
GeoCas9	NNNN CRAA		2	21	23	GTCATA GTTCCC CTGA	GAAA	TCAGGGTACTATGATAAGGG CTTTCTGCCTAAGGCAGACTG ACCCGCGGCGTTGGGGATCGC CTGTCGCCCGCTTTGGCGGG CATTCCCCATCCTT
iSpyMacCas9	NAAN	-3	2	19	21	GTTTTA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
NmeCas9	NNNN GAYT;N NNNGY TT;NN NNGAY	-3	2	20	24	GTTGTA GCTCCC TTTCTC ATTTCG	GAAA	CGAAATGAGAACCGTTGCTAC AATAAGGCCGTCTGAAAAGAT GTGCCGCAACGCTCTGCCCTT AAAGCTTCTGCTTAAGGGGC ATCGTTTA

	A;NNN NGTCT							
ScaCas9	NNG	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
ScaCas9- HiFi-Sc++	NNG	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9- 3var-NRRH	NRRH	-3	2	20	20	GTTTAA GAGCTA TGCTG	GAAA	CAGCATAGCAAGTTTAAATAA GGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGT GC
SpyCas9- 3var-NRTH	NRTH	-3	2	20	20	GTTTAA GAGCTA TGCTG	GAAA	CAGCATAGCAAGTTTAAATAA GGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGT GC
SpyCas9- 3var-NRCH	NRCH	-3	2	20	20	GTTTAA GAGCTA TGCTG	GAAA	CAGCATAGCAAGTTTAAATAA GGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCGAGTCGGT GC
SpyCas9-HF1	NGG	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9- QQR1	NAAG	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9-SpG	NGN	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9- VQR	NGAN	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC
SpyCas9- VRER	NGCG	-3	2	20	20	GTTTAA GAGCTA	GAAA	TAGCAAGTTAAAATAAGGCTA GTCCGTTATCAACTTGAAAAA GTGGCACCGAGTCGGTGC

SpyCas9- xCas	NG;GA A;GAT	-3	2	20	20	GTTTAA GAGCTA TGCTG	GAAA	CAGCATAGCAAGTTTAAATAA GGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCAGTCGGT GC
SpyCas9- xCas-NG	NG	-3	2	20	20	GTTTAA GAGCTA TGCTG	GAAA	CAGCATAGCAAGTTTAAATAA GGCTAGTCCGTTATCAACTTG AAAAAGTGGCACCAGTCGGT GC
St1Cas9- CNRZ1066	NNACA A	-3	2	20	20	GTCTTT GTACTC TG	GTAC	CAGAAGCTACAAAGATAAGGC TTCATGCCGAAATCAACACCCT GTCATTTTATGGCAGGGTGTT TT
St1Cas9- LMG1831	NNGCA A	-3	2	20	20	GTCTTT GTACTC TG	GTAC	CAGAAGCTACAAAGATAAGGC TTCATGCCGAAATCAACACCCT GTCATTTTATGGCAGGGTGTT TT
St1Cas9- MTH17CL39 6	NNAAA A	-3	2	20	20	GTCTTT GTACTC TG	GTAC	CAGAAGCTACAAAGATAAGGC TTCATGCCGAAATCAACACCCT GTCATTTTATGGCAGGGTGTT TT
St1Cas9- TH1477	NNGAA A	-3	2	20	20	GTCTTT GTACTC TG	GTAC	CAGAAGCTACAAAGATAAGGC TTCATGCCGAAATCAACACCCT GTCATTTTATGGCAGGGTGTT TT
sRGN3.1	NNGG		1	21	23	GTTTTA GTACTC TG	GAAA	CAGAATCTACTGAAACAAGAC AATATGTCGTGTTTATCCCATC AATTTATTGGTGGGATTTT
sRGN3.3	NNGG		1	21	23	GTTTTA GTACTC TG	GAAA	CAGAATCTACTGAAACAAGAC AATATGTCGTGTTTATCCCATC AATTTATTGGTGGGATTTT

Additionally, it is understood that terminal Us and Ts may optionally be added or removed from tracrRNA sequences and may be modified or unmodified when provided as RNA. Without wishing to be bound by example, versions of gRNA scaffold sequences alternative to those exemplified in Table 8 may also function with the different Cas9 enzymes or derivatives thereof exemplified in Table 4, e.g., alternate gRNA scaffold sequences with nucleotide additions, substitutions, or deletions, e.g., sequences with stem-loop structures added or

removed. It is contemplated herein that the gRNA scaffold sequences represent a component of gene modifying systems that can be similarly optimized for a given system, Cas-RT fusion polypeptide, indication, target mutation, template RNA, or delivery vehicle.

Herein, when an RNA sequence (e.g., a template RNA sequence) is said to comprise a particular sequence (e.g., a sequence of Table 8 or a portion thereof) that comprises thymine (T), it is of course understood that the RNA sequence may (and frequently does) comprise uracil (U) in place of T. For instance, the RNA sequence may comprise U at every position shown as T in the sequence in Table 8. More specifically, the present disclosure provides an RNA sequence according to every gRNA scaffold sequence of Table 8, wherein the RNA sequence has a U in place of each T in the sequence in Table 8.

Heterologous object sequence

A template RNA described herein may comprise a heterologous object sequence that the gene modifying polypeptide can use as a template for reverse transcription, to write a desired sequence into the target nucleic acid. In some embodiments, the heterologous object sequence comprises, from 5' to 3', a post-edit homology region, the mutation region, and a pre-edit homology region. Without wishing to be bound by theory, an RT performing reverse transcription on the template RNA first reverse transcribes the pre-edit homology region, then the mutation region, and then the post-edit homology region, thereby creating a DNA strand comprising the desired mutation with a homology region on either side.

In some embodiments, the heterologous object sequence is at least 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 120, 140, 160, 180, 200, 500, or 1,000 nucleotides (nts) in length, or at least 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 kilobases in length. In some embodiments, the heterologous object sequence is no more than 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 120, 140, 160, 180, 200, 500, 1,000, or 2000 nucleotides (nts) in length, or no more than 20, 15, 10, 9, 8, 7, 6, 5, 4, or 3 kilobases in length. In some embodiments, the heterologous object sequence is 30-1000, 40-1000, 50-1000,

60-1000, 70-1000, 74-1000, 75-1000, 76-1000, 77-1000, 78-1000, 79-1000, 80-1000, 85-1000, 90-1000, 100-1000, 120-1000, 140-1000, 160-1000, 180-1000, 200-1000, 500-1000, 30-500, 40-500, 50-500, 60-500, 70-500, 74-500, 75-500, 76-500, 77-500, 78-500, 79-500, 80-500, 85-500, 90-500, 100-500, 120-500, 140-500, 160-500, 180-500, 200-500, 30-200, 40-200, 50-200, 60-200, 70-200, 74-200, 75-200, 76-200, 77-200, 78-200, 79-200, 80-200, 85-200, 90-200, 100-200, 120-200, 140-200, 160-200, 180-200, 30-100, 40-100, 50-100, 60-100, 70-100, 74-100, 75-100, 76-100, 77-100, 78-100, 79-100, 80-100, 85-100, or 90-100 nucleotides (nts) in length, or 1-20, 1-15, 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-20, 2-15, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-20, 3-15, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-20, 4-15, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-20, 5-15, 5-10, 5-9, 5-8, 5-7, 5-6, 6-20, 6-15, 6-10, 6-9, 6-8, 6-7, 7-20, 7-15, 7-10, 7-9, 7-8, 8-20, 8-15, 8-10, 8-9, 9-20, 9-15, 9-10, 10-15, 10-20, or 15-20 kilobases in length. In some embodiments, the heterologous object sequence is 10-100, 10-90, 10-80, 10-70, 10-60, 10-50, 10-40, 10-30, or 10-20 nt in length, e.g., 10-80, 10-50, or 10-20 nt in length, e.g., about 10-20 nt in length. In some embodiments, the heterologous object sequence is 8-30, 9-25, 10-20, 11-16, or 12-15 nucleotides in length, e.g., is 11-16 nt in length. Without wishing to be bound by theory, in some embodiments, a larger insertion size, larger region of editing (e.g., the distance between a first edit/substitution and a second edit/substitution in the target region), and/or greater number of desired edits (e.g., mismatches of the heterologous object sequence to the target genome), may result in a longer optimal heterologous object sequence.

In certain embodiments, the template nucleic acid comprises a customized RNA sequence template which can be identified, designed, engineered and constructed to contain sequences altering or specifying host genome function, for example by introducing a heterologous coding region into a genome; affecting or causing exon structure/alternative splicing, e.g., leading to exon skipping of one or more exons; causing disruption of an endogenous gene, e.g., creating a genetic knockout; causing transcriptional activation of an endogenous gene; causing epigenetic regulation of an endogenous DNA; causing up-regulation of one or more operably linked genes, e.g., leading to gene activation or overexpression; causing down-regulation of one or more operably linked genes, e.g., creating a genetic knock-down; etc. In certain embodiments, a customized RNA sequence template can be engineered to contain sequences coding for exons and/or transgenes, provide binding sites for transcription factor activators, repressors, enhancers, etc., and combinations thereof. In some embodiments, a customized template can be engineered

to encode a nucleic acid or peptide tag to be expressed in an endogenous RNA transcript or endogenous protein operably linked to the target site. In other embodiments, the coding sequence can be further customized with splice donor sites, splice acceptor sites, or poly-A tails.

The template nucleic acid (e.g., template RNA) of the system typically comprises an object sequence (e.g., a heterologous object sequence) for writing a desired sequence into a target DNA. The object sequence may be coding or non-coding. The template nucleic acid (e.g., template RNA) can be designed to result in insertions, mutations, or deletions at the target DNA locus. In some embodiments, the template nucleic acid (e.g., template RNA) may be designed to cause an insertion in the target DNA. For example, the template nucleic acid (e.g., template RNA) may contain a heterologous sequence, wherein the reverse transcription will result in insertion of the heterologous sequence into the target DNA. In other embodiments, the RNA template may be designed to introduce a deletion into the target DNA. For example, the template nucleic acid (e.g., template RNA) may match the target DNA upstream and downstream of the desired deletion, wherein the reverse transcription will result in the copying of the upstream and downstream sequences from the template nucleic acid (e.g., template RNA) without the intervening sequence, e.g., causing deletion of the intervening sequence. In other embodiments, the template nucleic acid (e.g., template RNA) may be designed to introduce an edit into the target DNA. For example, the template RNA may match the target DNA sequence with the exception of one or more nucleotides, wherein the reverse transcription will result in the copying of these edits into the target DNA, e.g., resulting in mutations, e.g., transition or transversion mutations.

In some embodiments, writing of an object sequence into a target site results in the substitution of nucleotides, e.g., where the full length of the object sequence corresponds to a matching length of the target site with one or more mismatched bases. In some embodiments, a heterologous object sequence may be designed such that a combination of sequence alterations may occur, e.g., a simultaneous addition and deletion, addition and substitution, or deletion and substitution.

In some embodiments, the heterologous object sequence may contain an open reading frame or a fragment of an open reading frame. In some embodiments the heterologous object sequence has a Kozak sequence. In some embodiments the heterologous object sequence has an internal ribosome entry site. In some embodiments the heterologous object sequence has a self-

cleaving peptide such as a T2A or P2A site. In some embodiments the heterologous object sequence has a start codon. In some embodiments the template RNA has a splice acceptor site. In some embodiments the template RNA has a splice donor site. Exemplary splice acceptor and splice donor sites are described in WO2016044416, incorporated herein by reference in its entirety. Exemplary splice acceptor site sequences are known to those of skill in the art. In some 5 embodiments the template RNA has a microRNA binding site downstream of the stop codon. In some embodiments the template RNA has a polyA tail downstream of the stop codon of an open reading frame. In some embodiments the template RNA comprises one or more exons. In some embodiments the template RNA comprises one or more introns. In some embodiments the 10 template RNA comprises a eukaryotic transcriptional terminator. In some embodiments the template RNA comprises an enhanced translation element or a translation enhancing element. In some embodiments the RNA comprises the human T-cell leukemia virus (HTLV-1) R region. In some embodiments the RNA comprises a posttranscriptional regulatory element that enhances nuclear export, such as that of Hepatitis B Virus (HPRE) or Woodchuck Hepatitis Virus 15 (WPRE).

In some embodiments, the heterologous object sequence may contain a non-coding sequence. For example, the template nucleic acid (e.g., template RNA) may comprise a regulatory element, e.g., a promoter or enhancer sequence or miRNA binding site. In some 20 embodiments, integration of the object sequence at a target site will result in upregulation of an endogenous gene. In some embodiments, integration of the object sequence at a target site will result in downregulation of an endogenous gene. In some embodiments the template nucleic acid (e.g., template RNA) comprises a tissue specific promoter or enhancer, each of which may be unidirectional or bidirectional. In some embodiments the promoter is an RNA polymerase I promoter, RNA polymerase II promoter, or RNA polymerase III promoter. In some 25 embodiments the promoter comprises a TATA element. In some embodiments the promoter comprises a B recognition element. In some embodiments the promoter has one or more binding sites for transcription factors.

In some embodiments, the template nucleic acid (e.g., template RNA) comprises a site that coordinates epigenetic modification. In some embodiments, the template nucleic acid (e.g., 30 template RNA) comprises a chromatin insulator. For example, the template nucleic acid (e.g., template RNA) comprises a CTCF site or a site targeted for DNA methylation.

In some embodiments, the template nucleic acid (e.g., template RNA) comprises a gene expression unit composed of at least one regulatory region operably linked to an effector sequence. The effector sequence may be a sequence that is transcribed into RNA (e.g., a coding sequence or a non-coding sequence such as a sequence encoding a micro RNA).

5 In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is inserted into a target genome in an endogenous intron. In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is inserted into a target genome and thereby acts as a new exon. In some embodiments, the insertion of the heterologous object sequence into the target genome results in replacement of
10 a natural exon or the skipping of a natural exon.

In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is inserted into the target genome in a genomic safe harbor site, such as AAVS1, CCR5, ROSA26, or albumin locus. In some embodiments, a gene modifying is used to integrate a CAR into the T-cell receptor α constant (*TRAC*) locus (Eyquem et al Nature 543, 113-
15 117 (2017)). In some embodiments, a gene modifying system is used to integrate a CAR into a T-cell receptor β constant (*TRBC*) locus. Many other safe harbors have been identified by computational approaches (Pellenz et al Hum Gen Ther 30, 814-828 (2019)) and could be used for gene modifying system-mediated integration. In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is added to the genome in an
20 intergenic or intragenic region. In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is added to the genome 5' or 3' within 0.1 kb, 0.25 kb, 0.5 kb, 0.75 kb, 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 7.5 kb, 10 kb, 15 kb, 20 kb, 25 kb, 50, 75 kb, or 100 kb of an endogenous active gene. In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) is added to the genome 5' or 3' within 0.1 kb,
25 0.25 kb, 0.5 kb, 0.75 kb, 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 7.5 kb, 10 kb, 15 kb, 20 kb, 25 kb, 50, 75 kb, or 100 kb of an endogenous promoter or enhancer. In some embodiments, the heterologous object sequence of the template nucleic acid (e.g., template RNA) can be, e.g., 50-50,000 base pairs (e.g., between 50-40,000 bp, between 500-30,000 bp between 500-20,000 bp, between 100-15,000 bp, between 500-10,000 bp, between 50-10,000 bp, between 50-5,000 bp).

30 The template nucleic acid (e.g., template RNA) can be designed to result in insertions, mutations, or deletions at the target DNA locus. In some embodiments, the template nucleic acid

(e.g., template RNA) may be designed to cause an insertion in the target DNA. For example, the template nucleic acid (e.g., template RNA) may contain a heterologous object sequence, wherein the reverse transcription will result in insertion of the heterologous object sequence into the target DNA. In other embodiments, the RNA template may be designed to write a deletion into the target DNA. For example, the template nucleic acid (e.g., template RNA) may match the target DNA upstream and downstream of the desired deletion, wherein the reverse transcription will result in the copying of the upstream and downstream sequences from the template nucleic acid (e.g., template RNA) without the intervening sequence, e.g., causing deletion of the intervening sequence. In other embodiments, the template nucleic acid (e.g., template RNA) may be designed to write an edit into the target DNA. For example, the template RNA may match the target DNA sequence with the exception of one or more nucleotides, wherein the reverse transcription will result in the copying of these edits into the target DNA, e.g., resulting in mutations, e.g., transition or transversion mutations.

In some embodiments, the pre-edit homology domain comprises a nucleic acid sequence having 100% sequence identity with a nucleic acid sequence comprised in a target nucleic acid molecule.

In some embodiments, the post-edit homology domain comprises a nucleic acid sequence having 100% sequence identity with a nucleic acid sequence comprised in a target nucleic acid molecule.

PBS sequence

In some embodiments, a template nucleic acid (e.g., template RNA) comprises a PBS sequence. In some embodiments, a PBS sequence is disposed 3' of the heterologous object sequence and is complementary to a sequence adjacent to a site to be modified by a system described herein, or comprises no more than 1, 2, 3, 4, or 5 mismatches to a sequence complementary to the sequence adjacent to a site to be modified by the system/gene modifying polypeptide. In some embodiments, the PBS sequence binds within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides of a nick site in the target nucleic acid molecule. In some embodiments, binding of the PBS sequence to the target nucleic acid molecule permits initiation of target-primed reverse transcription (TPRT), e.g., with the 3' homology domain acting as a primer for TPRT. In some embodiments, the PBS sequence is 3-5, 5-10, 10-30, 10-25, 10-20, 10-19, 10-18, 10-17, 10-16,

10-15, 10-14, 10-13, 10-12, 10-11, 11-30, 11-25, 11-20, 11-19, 11-18, 11-17, 11-16, 11-15, 11-14, 11-13, 11-12, 12-30, 12-25, 12-20, 12-19, 12-18, 12-17, 12-16, 12-15, 12-14, 12-13, 13-30, 13-25, 13-20, 13-19, 13-18, 13-17, 13-16, 13-15, 13-14, 14-30, 14-25, 14-20, 14-19, 14-18, 14-17, 14-16, 14-15, 15-30, 15-25, 15-20, 15-19, 15-18, 15-17, 15-16, 16-30, 16-25, 16-20, 16-19, 5 16-18, 16-17, 17-30, 17-25, 17-20, 17-19, 17-18, 18-30, 18-25, 18-20, 18-19, 19-30, 19-25, 19-20, 20-30, 20-25, or 25-30 nucleotides in length, e.g., 10-17, 12-16, or 12-14 nucleotides in length. In some embodiments, the PBS sequence is 5-20, 8-16, 8-14, 8-13, 9-13, 9-12, or 10-12 nucleotides in length, e.g., 9-12 nucleotides in length.

The template nucleic acid (e.g., template RNA) may have some homology to the target DNA. In some embodiments, the template nucleic acid (e.g., template RNA) PBS sequence domain may serve as an annealing region to the target DNA, such that the target DNA is positioned to prime the reverse transcription of the template nucleic acid (e.g., template RNA). In some embodiments the template nucleic acid (e.g., template RNA) has at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 15 175, 200 or more bases of exact homology to the target DNA at the 3' end of the RNA. In some embodiments the template nucleic acid (e.g., template RNA) has at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 175, 200 or more bases of at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% homology to the target DNA, e.g., at the 5' end of the template nucleic acid (e.g., template RNA). 20

gRNAs with inducible activity

In some embodiments, a gRNA described herein (e.g., a gRNA that is part of a template RNA or a gRNA used for second strand nicking) has inducible activity. Inducible activity may be achieved by the template nucleic acid, e.g., template RNA, further comprising (in addition to 25 the gRNA) a blocking domain, wherein the sequence of a portion of or all of the blocking domain is at least partially complementary to a portion or all of the gRNA. The blocking domain is thus capable of hybridizing or substantially hybridizing to a portion of or all of the gRNA. In some embodiments, the blocking domain and inducibly active gRNA are disposed on the template nucleic acid, e.g., template RNA, such that the gRNA can adopt a first conformation 30 where the blocking domain is hybridized or substantially hybridized to the gRNA, and a second

conformation where the blocking domain is not hybridized or not substantially hybridized to the gRNA. In some embodiments, in the first conformation the gRNA is unable to bind to the gene modifying polypeptide (e.g., the template nucleic acid binding domain, DNA binding domain, or endonuclease domain (e.g., a CRISPR/Cas protein)) or binds with substantially decreased
5 affinity compared to an otherwise similar template RNA lacking the blocking domain. In some embodiments, in the second conformation the gRNA is able to bind to the gene modifying polypeptide (e.g., the template nucleic acid binding domain, DNA binding domain, or endonuclease domain (e.g., a CRISPR/Cas protein)). In some embodiments, whether the gRNA is in the first or second conformation can influence whether the DNA binding or endonuclease
10 activities of the gene modifying polypeptide (e.g., of the CRISPR/Cas protein the gene modifying polypeptide comprises) are active.

In some embodiments, the gRNA that coordinates the second nick has inducible activity. In some embodiments, the gRNA that coordinates the second nick is induced after the template is reverse transcribed. In some embodiments, hybridization of the gRNA to the blocking domain
15 can be disrupted using an opener molecule. In some embodiments, an opener molecule comprises an agent that binds to a portion or all of the gRNA or blocking domain and inhibits hybridization of the gRNA to the blocking domain. In some embodiments, the opener molecule comprises a nucleic acid, e.g., comprising a sequence that is partially or wholly complementary to the gRNA, blocking domain, or both. By choosing or designing an appropriate opener
20 molecule, providing the opener molecule can promote a change in the conformation of the gRNA such that it can associate with a CRISPR/Cas protein and provide the associated functions of the CRISPR/Cas protein (e.g., DNA binding and/or endonuclease activity). Without wishing to be bound by theory, providing the opener molecule at a selected time and/or location may allow for spatial and temporal control of the activity of the gRNA, CRISPR/Cas protein, or gene
25 modifying system comprising the same. In some embodiments, the opener molecule is exogenous to the cell comprising the gene modifying polypeptide and or template nucleic acid. In some embodiments, the opener molecule comprises an endogenous agent (e.g., endogenous to the cell comprising the gene modifying polypeptide and or template nucleic acid comprising the gRNA and blocking domain). For example, an inducible gRNA, blocking domain, and opener
30 molecule may be chosen such that the opener molecule is an endogenous agent expressed in a target cell or tissue, e.g., thereby ensuring activity of a gene modifying system in the target cell

or tissue. As a further example, an inducible gRNA, blocking domain, and opener molecule may be chosen such that the opener molecule is absent or not substantially expressed in one or more non-target cells or tissues, e.g., thereby ensuring that activity of a gene modifying system does not occur or substantially occur in the one or more non-target cells or tissues, or occurs at a reduced level compared to a target cell or tissue. Exemplary blocking domains, opener molecules, and uses thereof are described in PCT App. Publication WO2020044039A1, which is incorporated herein by reference in its entirety. In some embodiments, the template nucleic acid, e.g., template RNA, may comprise one or more sequences or structures for binding by one or more components of a gene modifying polypeptide, e.g., by a reverse transcriptase or RNA binding domain, and a gRNA. In some embodiments, the gRNA facilitates interaction with the template nucleic acid binding domain (e.g., RNA binding domain) of the gene modifying polypeptide. In some embodiments, the gRNA directs the gene modifying polypeptide to the matching target sequence, e.g., in a target cell genome.

Circular RNAs and Ribozymes in Gene modifying Systems

It is contemplated that it may be useful to employ circular and/or linear RNA states during the formulation, delivery, or gene modifying reaction within the target cell. Thus, in some embodiments of any of the aspects described herein, a gene modifying system comprises one or more circular RNAs (circRNAs). In some embodiments of any of the aspects described herein, a gene modifying system comprises one or more linear RNAs. In some embodiments, a nucleic acid as described herein (e.g., a template nucleic acid, a nucleic acid molecule encoding a gene modifying polypeptide, or both) is a circRNA. In some embodiments, a circular RNA molecule encodes the gene modifying polypeptide. In some embodiments, the circRNA molecule encoding the gene modifying polypeptide is delivered to a host cell. In some embodiments, a circular RNA molecule encodes a recombinase, e.g., as described herein. In some embodiments, the circRNA molecule encoding the recombinase is delivered to a host cell. In some embodiments, the circRNA molecule encoding the gene modifying polypeptide is linearized (e.g., in the host cell, e.g., in the nucleus of the host cell) prior to translation.

Circular RNAs (circRNAs) have been found to occur naturally in cells and have been found to have diverse functions, including both non-coding and protein coding roles in human cells. It has been shown that a circRNA can be engineered by incorporating a self-splicing intron

into an RNA molecule (or DNA encoding the RNA molecule) that results in circularization of the RNA, and that an engineered circRNA can have enhanced protein production and stability (Wesselhoeft et al. *Nature Communications* 2018). In some embodiments, the gene modifying polypeptide is encoded as circRNA. In certain embodiments, the template nucleic acid is a
5 DNA, such as a dsDNA or ssDNA. In certain embodiments, the circDNA comprises a template RNA.

In some embodiments, the circRNA comprises one or more ribozyme sequences. In some embodiments, the ribozyme sequence is activated for autocleavage, e.g., in a host cell, e.g., thereby resulting in linearization of the circRNA. In some embodiments, the ribozyme is
10 activated when the concentration of magnesium reaches a sufficient level for cleavage, e.g., in a host cell. In some embodiments the circRNA is maintained in a low magnesium environment prior to delivery to the host cell. In some embodiments, the ribozyme is a protein-responsive ribozyme. In some embodiments, the ribozyme is a nucleic acid-responsive ribozyme. In some
15 embodiments, the circRNA comprises a cleavage site. In some embodiments, the circRNA comprises a second cleavage site.

In some embodiments, the circRNA is linearized in the nucleus of a target cell. In some embodiments, linearization of a circRNA in the nucleus of a cell involves components present in the nucleus of the cell, e.g., to activate a cleavage event. In some embodiments, a ribozyme, e.g., a ribozyme from a B2 or ALU element, that is responsive to a nuclear element, e.g., a nuclear
20 protein, e.g., a genome-interacting protein, e.g., an epigenetic modifier, e.g., EZH2, is incorporated into a circRNA, e.g., of a gene modifying system. In some embodiments, nuclear localization of the circRNA results in an increase in autocatalytic activity of the ribozyme and linearization of the circRNA.

In some embodiments, the ribozyme is heterologous to one or more of the other
25 components of the gene modifying system. In some embodiments, an inducible ribozyme (e.g., in a circRNA as described herein) is created synthetically, for example, by utilizing a protein ligand-responsive aptamer design. A system for utilizing the satellite RNA of tobacco ringspot virus hammerhead ribozyme with an MS2 coat protein aptamer has been described (Kennedy et al. *Nucleic Acids Res* 42(19):12306-12321 (2014), incorporated herein by reference in its
30 entirety) that results in activation of the ribozyme activity in the presence of the MS2 coat

protein. In embodiments, such a system responds to protein ligand localized to the cytoplasm or the nucleus. In some embodiments the protein ligand is not MS2. Methods for generating RNA aptamers to target ligands have been described, for example, based on the systematic evolution of ligands by exponential enrichment (SELEX) (Tuerk and Gold, Science 249(4968):505-510
5 (1990); Ellington and Szostak, Nature 346(6287):818-822 (1990); the methods of each of which are incorporated herein by reference) and have, in some instances, been aided by *in silico* design (Bell et al. PNAS 117(15):8486-8493, the methods of which are incorporated herein by reference). Thus, in some embodiments, an aptamer for a target ligand is generated and incorporated into a synthetic ribozyme system, e.g., to trigger ribozyme-mediated cleavage and
10 circRNA linearization, e.g., in the presence of the protein ligand. In some embodiments, circRNA linearization is triggered in the cytoplasm, e.g., using an aptamer that associates with a ligand in the cytoplasm. In some embodiments, circRNA linearization is triggered in the nucleus, e.g., using an aptamer that associates with a ligand in the nucleus. In embodiments, the ligand in the nucleus comprises an epigenetic modifier or a transcription factor. In some embodiments the
15 ligand that triggers linearization is present at higher levels in on-target cells than off-target cells.

It is further contemplated that a nucleic acid-responsive ribozyme system can be employed for circRNA linearization. For example, biosensors that sense defined target nucleic acid molecules to trigger ribozyme activation are described, e.g., in Penchovsky (Biotechnology Advances 32(5):1015-1027 (2014), incorporated herein by reference). By these methods, a
20 ribozyme naturally folds into an inactive state and is only activated in the presence of a defined target nucleic acid molecule (e.g., an RNA molecule). In some embodiments, a circRNA of a gene modifying system comprises a nucleic acid-responsive ribozyme that is activated in the presence of a defined target nucleic acid, e.g., an RNA, e.g., an mRNA, miRNA, guide RNA, gRNA, sgRNA, ncRNA, lncRNA, tRNA, snRNA, or mtRNA. In some embodiments the nucleic
25 acid that triggers linearization is present at higher levels in on-target cells than off-target cells.

In some embodiments of any of the aspects herein, a gene modifying system incorporates one or more ribozymes with inducible specificity to a target tissue or target cell of interest, e.g., a ribozyme that is activated by a ligand or nucleic acid present at higher levels in a target tissue or target cell of interest. In some embodiments, the gene modifying system incorporates a
30 ribozyme with inducible specificity to a subcellular compartment, e.g., the nucleus, nucleolus,

cytoplasm, or mitochondria. In some embodiments, the ribozyme that is activated by a ligand or nucleic acid present at higher levels in the target subcellular compartment. In some
embodiments, an RNA component of a gene modifying system is provided as circRNA, e.g., that
is activated by linearization. In some embodiments, linearization of a circRNA encoding a gene
5 modifying polypeptide activates the molecule for translation. In some embodiments, a signal that
activates a circRNA component of a gene modifying system is present at higher levels in on-
target cells or tissues, e.g., such that the system is specifically activated in these cells.

In some embodiments, an RNA component of a gene modifying system is provided as a
circRNA that is inactivated by linearization. In some embodiments, a circRNA encoding the
10 gene modifying polypeptide is inactivated by cleavage and degradation. In some embodiments, a
circRNA encoding the gene modifying polypeptide is inactivated by cleavage that separates a
translation signal from the coding sequence of the polypeptide. In some embodiments, a signal
that inactivates a circRNA component of a gene modifying system is present at higher levels in
off-target cells or tissues, such that the system is specifically inactivated in these cells.

15 ***Target Nucleic Acid Site***

In some embodiments, after gene modification, the target site surrounding the edited
sequence contains a limited number of insertions or deletions, for example, in less than about
50% or 10% of editing events, e.g., as determined by long-read amplicon sequencing of the
target site, e.g., as described in Karst et al. (2020) *bioRxiv* doi.org/10.1101/645903 (incorporated
20 by reference herein in its entirety). In some embodiments, the target site does not show multiple
consecutive editing events, e.g., head-to-tail or head-to-head duplications, e.g., as determined by
long-read amplicon sequencing of the target site, e.g., as described in Karst et al. *bioRxiv*
doi.org/10.1101/645903 (2020) (incorporated herein by reference in its entirety). In some
embodiments, the target site contains an integrated sequence corresponding to the template RNA.
25 In some embodiments, the target site does not contain insertions resulting from endogenous RNA
in more than about 1% or 10% of events, e.g., as determined by long-read amplicon sequencing
of the target site, e.g., as described in Karst et al. *bioRxiv* doi.org/10.1101/645903 (2020)
(incorporated herein by reference in its entirety). In some embodiments, the target site contains
the integrated sequence corresponding to the template RNA.

In certain aspects of the present invention, the host DNA-binding site integrated into by the gene modifying system can be in a gene, in an intron, in an exon, an ORF, outside of a coding region of any gene, in a regulatory region of a gene, or outside of a regulatory region of a gene. In other aspects, the polypeptide may bind to one or more than one host DNA sequence.

5 In some embodiments, a gene modifying system is used to edit a target locus in multiple alleles. In some embodiments, a gene modifying system is designed to edit a specific allele. For example, a gene modifying polypeptide may be directed to a specific sequence that is only present on one allele, e.g., comprises a template RNA with homology to a target allele, e.g., a gRNA or annealing domain, but not to a second cognate allele. In some embodiments, a gene
10 modifying system can alter a haplotype-specific allele. In some embodiments, a gene modifying system that targets a specific allele preferentially targets that allele, e.g., has at least a 2, 4, 6, 8, or 10-fold preference for a target allele.

Second Strand Nicking

In some embodiments, a gene modifying system described herein comprises a nickase
15 activity (e.g., in the gene modifying polypeptide) that nicks the first strand, and a nickase activity (e.g., in a polypeptide separate from the gene modifying polypeptide) that nicks the second strand of target DNA. As discussed herein, without wishing to be bound by theory, nicking of the first strand of the target site DNA is thought to provide a 3' OH that can be used by an RT domain to reverse transcribe a sequence of a template RNA, e.g., a heterologous object sequence.
20 Without wishing to be bound by theory, it is thought that introducing an additional nick to the second strand may bias the cellular DNA repair machinery to adopt the heterologous object sequence-based sequence more frequently than the original genomic sequence. In some embodiments, the additional nick to the second strand is made by the same endonuclease domain (e.g., nickase domain) as the nick to the first strand. In some embodiments, the same gene
25 modifying polypeptide performs both the nick to the first strand and the nick to the second strand. In some embodiments, the gene modifying polypeptide comprises a CRISPR/Cas domain and the additional nick to the second strand is directed by an additional nucleic acid, e.g., comprising a second gRNA directing the CRISPR/Cas domain to nick the second strand. In other embodiments, the additional second strand nick is made by a different endonuclease
30 domain (e.g., nickase domain) than the nick to the first strand. In some embodiments, that

different endonuclease domain is situated in an additional polypeptide (e.g., a system of the invention further comprises the additional polypeptide), separate from the gene modifying polypeptide. In some embodiments, the additional polypeptide comprises an endonuclease domain (e.g., nickase domain) described herein. In some embodiments, the additional
5 polypeptide comprises a DNA binding domain, e.g., described herein.

It is contemplated herein that the position at which the second strand nick occurs relative to the first strand nick may influence the extent to which one or more of: desired gene modifying DNA modifications are obtained, undesired double-strand breaks (DSBs) occur, undesired insertions occur, or undesired deletions occur. Without wishing to be bound by theory, second
10 strand nicking may occur in two general orientations: inward nicks and outward nicks.

In some embodiments, in the inward nick orientation, the RT domain polymerizes (e.g., using the template RNA (e.g., the heterologous object sequence)) away from the second strand nick. In some embodiments, in the inward nick orientation, the location of the nick to the first strand and the location of the nick to the second strand are positioned between the first PAM site
15 and second PAM site (e.g., in a scenario wherein both nicks are made by a polypeptide (e.g., a gene modifying polypeptide) comprising a CRISPR/Cas domain). In some embodiments, in the inward nick orientation, the location of the nick to the first strand and the location of the nick to the second strand are between the sites where the polypeptide and the additional polypeptide bind to the target DNA. In some embodiments, in the inward nick orientation, the location of the
20 nick to the second strand is positioned on the same side of the binding sites of the polypeptide and additional polypeptide relative to the location of the nick to the first strand. In some embodiments, in the inward nick orientation, the location of the nick to the first strand and the location of the nick to the second strand are positioned between the PAM site and the site at a distance from the target site.

25 An example of a gene modifying system that provides an inward nick orientation comprises a gene modifying polypeptide comprising a CRISPR/Cas domain, a template RNA comprising a gRNA that directs nicking of the target site DNA on the first strand, and an additional nucleic acid comprising an additional gRNA that directs nicking at a site a distance from the location of the first nick, wherein the location of the first nick and the location of the
30 second nick are between the PAM sites of the sites to which the two gRNAs direct the gene modifying polypeptide. As a further example, another gene modifying system that provides an

inward nick orientation comprises a gene modifying polypeptide comprising a zinc finger molecule and a first nickase domain wherein the zinc finger molecule binds to the target DNA in a manner that directs the first nickase domain to nick the first strand of the target site; an additional polypeptide comprising a CRISPR/Cas domain, and an additional nucleic acid
5 comprising a gRNA that directs the additional polypeptide to nick a site a distance from the target site DNA on the second strand, wherein the location of the first nick and the location of the second nick are between the PAM site and the site to which the zinc finger molecule binds. As a further example, another gene modifying system that provides an inward nick orientation comprises a gene modifying polypeptide comprising a zinc finger molecule and a first nickase
10 domain wherein the zinc finger molecule binds to the target DNA in a manner that directs the first nickase domain to nick the first strand of the target site; an additional polypeptide comprising a TAL effector molecule and a second nickase domain wherein the TAL effector molecule binds to a site a distance from the target site in a manner that directs the additional polypeptide to nick the second strand, wherein the location of the first nick and the location of
15 the second nick are between the site to which the TAL effector molecule binds and the site to which the zinc finger molecule binds.

In some embodiments, in the outward nick orientation, the RT domain polymerizes (e.g., using the template RNA (e.g., the heterologous object sequence)) toward the second strand nick. In some embodiments, in the inward nick orientation when both the first and second nicks are
20 made by a polypeptide comprising a CRISPR/Cas domain (e.g., a gene modifying polypeptide), the first PAM site and second PAM site are positioned between the location of the nick to the first strand and the location of the nick to the second strand. In some embodiments, in the inward nick orientation, the polypeptide (e.g., the gene modifying polypeptide) and the additional polypeptide bind to sites on the target DNA between the location of the nick to the
25 first strand and the location of the nick to the second. In some embodiments, in the inward nick orientation, the location of the nick to the second strand is positioned on the opposite side of the binding sites of the polypeptide and additional polypeptide relative to the location of the nick to the first strand. In some embodiments, in the inward orientation, the PAM site and the site at a distance from the target site are positioned between the location of the nick to the first strand and
30 the location of the nick to the second strand.

An example of a gene modifying system that provides an outward nick orientation comprises a gene modifying polypeptide comprising a CRISPR/Cas domain, a template RNA comprising a gRNA that directs nicking of the target site DNA on the first strand, and an additional nucleic acid comprising an additional gRNA that directs nicking at a site a distance
5 from the location of the first nick, wherein the location of the first nick and the location of the second nick are outside of the PAM sites of the sites to which the two gRNAs direct the gene modifying polypeptide (i.e., the PAM sites are between the location of the first nick and the location of the second nick). As a further example, another gene modifying system that provides an outward nick orientation comprises a gene modifying polypeptide comprising a zinc finger
10 molecule and a first nickase domain wherein the zinc finger molecule binds to the target DNA in a manner that directs the first nickase domain to nick the first strand of the target site; an additional polypeptide comprising a CRISPR/Cas domain, and an additional nucleic acid comprising a gRNA that directs the additional polypeptide to nick a site a distance from the target site DNA on the second strand, wherein the location of the first nick and the location of
15 the second nick are outside the PAM site and the site to which the zinc finger molecule binds (i.e., the PAM site and the site to which the zinc finger molecule binds are between the location of the first nick and the location of the second nick). As a further example, another gene modifying system that provides an outward nick orientation comprises a gene modifying polypeptide comprising a zinc finger molecule and a first nickase domain wherein the zinc finger
20 molecule binds to the target DNA in a manner that directs the first nickase domain to nick the first strand of the target site; an additional polypeptide comprising a TAL effector molecule and a second nickase domain wherein the TAL effector molecule binds to a site a distance from the target site in a manner that directs the additional polypeptide to nick the second strand, wherein the location of the first nick and the location of the second nick are outside the site to which the
25 TAL effector molecule binds and the site to which the zinc finger molecule binds (i.e., the site to which the TAL effector molecule binds and the site to which the zinc finger molecule binds are between the location of the first nick and the location of the second nick).

Without wishing to be bound by theory, it is thought that, for gene modifying systems where a second strand nick is provided, an outward nick orientation is preferred in some
30 embodiments. As is described herein, an inward nick may produce a higher number of double-strand breaks (DSBs) than an outward nick orientation. DSBs may be recognized by the DSB

repair pathways in the nucleus of a cell, which can result in undesired insertions and deletions. An outward nick orientation may provide a decreased risk of DSB formation, and a corresponding lower amount of undesired insertions and deletions. In some embodiments, undesired insertions and deletions are insertions and deletions not encoded by the heterologous object sequence, e.g., an insertion or deletion produced by the double-strand break repair pathway unrelated to the modification encoded by the heterologous object sequence. In some embodiments, a desired gene modification comprises a change to the target DNA (e.g., a substitution, insertion, or deletion) encoded by the heterologous object sequence (e.g., and achieved by the gene modifying writing the heterologous object sequence into the target site). In some embodiments, the first strand nick and the second strand nick are in an outward orientation.

In addition, the distance between the first strand nick and second strand nick may influence the extent to which one or more of: desired gene modifying system DNA modifications are obtained, undesired double-strand breaks (DSBs) occur, undesired insertions occur, or undesired deletions occur. Without wishing to be bound by theory, it is thought the second strand nick benefit, the biasing of DNA repair toward incorporation of the heterologous object sequence into the target DNA, increases as the distance between the first strand nick and second strand nick decreases. However, it is thought that the risk of DSB formation also increases as the distance between the first strand nick and second strand nick decreases. Correspondingly, it is thought that the number of undesired insertions and/or deletions may increase as the distance between the first strand nick and second strand nick decreases. In some embodiments, the distance between the first strand nick and second strand nick is chosen to balance the benefit of biasing DNA repair toward incorporation of the heterologous object sequence into the target DNA and the risk of DSB formation and of undesired deletions and/or insertions. In some embodiments, a system where the first strand nick and the second strand nick are at least a threshold distance apart has an increased level of desired gene modifying system modification outcomes, a decreased level of undesired deletions, and/or a decreased level of undesired insertions relative to an otherwise similar inward nick orientation system where the first nick and the second nick are less than the a threshold distance apart. In some embodiments the threshold distance(s) is given below.

In some embodiments, the first nick and the second nick are at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or

200 nucleotides apart. In some embodiments, the first nick and the second nick are no more than 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, or 250 nucleotides apart. In some embodiments, the first nick and the second nick are 20-200, 30-200, 40-200, 50-200, 60-200, 70-200, 80-200, 90-200, 100-200, 110-200, 120-
5 200, 130-200, 140-200, 150-200, 160-200, 170-200, 180-200, 190-200, 20-190, 30-190, 40-190, 50-190, 60-190, 70-190, 80-190, 90-190, 100-190, 110-190, 120-190, 130-190, 140-190, 150-190, 160-190, 170-190, 180-190, 20-180, 30-180, 40-180, 50-180, 60-180, 70-180, 80-180, 90-180, 100-180, 110-180, 120-180, 130-180, 140-180, 150-180, 160-180, 170-180, 20-170, 30-170, 40-170, 50-170, 60-170, 70-170, 80-170, 90-170, 100-170, 110-170, 120-170, 130-170,
10 140-170, 150-170, 160-170, 20-160, 30-160, 40-160, 50-160, 60-160, 70-160, 80-160, 90-160, 100-160, 110-160, 120-160, 130-160, 140-160, 150-160, 20-150, 30-150, 40-150, 50-150, 60-150, 70-150, 80-150, 90-150, 100-150, 110-150, 120-150, 130-150, 140-150, 20-140, 30-140, 40-140, 50-140, 60-140, 70-140, 80-140, 90-140, 100-140, 110-140, 120-140, 130-140, 20-130, 30-130, 40-130, 50-130, 60-130, 70-130, 80-130, 90-130, 100-130, 110-130, 120-130, 20-120,
15 30-120, 40-120, 50-120, 60-120, 70-120, 80-120, 90-120, 100-120, 110-120, 20-110, 30-110, 40-110, 50-110, 60-110, 70-110, 80-110, 90-110, 100-110, 20-100, 30-100, 40-100, 50-100, 60-100, 70-100, 80-100, 90-100, 20-90, 30-90, 40-90, 50-90, 60-90, 70-90, 80-90, 20-80, 30-80, 40-80, 50-80, 60-80, 70-80, 20-70, 30-70, 40-70, 50-70, 60-70, 20-60, 30-60, 40-60, 50-60, 20-50, 30-50, 40-50, 20-40, 30-40, or 20-30 nucleotides apart. In some embodiments, the first nick and the
20 second nick are 40-100 nucleotides apart.

Without wishing to be bound by theory, it is thought that, for gene modifying systems where a second strand nick is provided and an inward nick orientation is selected, increasing the distance between the first strand nick and second strand nick may be preferred. As is described herein, an inward nick orientation may produce a higher number of DSBs than an outward nick
25 orientation, and may result in a higher amount of undesired insertions and deletions than an outward nick orientation, but increasing the distance between the nicks may mitigate that increase in DSBs, undesired deletions, and/or undesired insertions. In some embodiments, an inward nick orientation wherein the first nick and the second nick are at least a threshold distance apart has an increased level of desired gene modifying system modification outcomes, a
30 decreased level of undesired deletions, and/or a decreased level of undesired insertions relative to an otherwise similar inward nick orientation system where the first nick and the second nick are

less than the a threshold distance apart. In some embodiments the threshold distance is given below.

In some embodiments, the first strand nick and the second strand nick are in an inward orientation. In some embodiments, the first strand nick and the second strand nick are in an inward orientation and the first strand nick and second strand nick are at least 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 350, 400, 450, or 500 nucleotides apart, e.g., at least 100 nucleotides apart, (and optionally no more than 500, 400, 300, 200, 190, 180, 170, 160, 150, 140, 130, or 120 nucleotides apart). In some embodiments, the first strand nick and the second strand nick are in an inward orientation and the first strand nick and second strand nick are 100-200, 110-200, 120-200, 130-200, 140-200, 150-200, 160-200, 170-200, 180-200, 190-200, 100-190, 110-190, 120-190, 130-190, 140-190, 150-190, 160-190, 170-190, 180-190, 100-180, 110-180, 120-180, 130-180, 140-180, 150-180, 160-180, 170-180, 100-170, 110-170, 120-170, 130-170, 140-170, 150-170, 160-170, 100-160, 110-160, 120-160, 130-160, 140-160, 150-160, 100-150, 110-150, 120-150, 130-150, 140-150, 100-140, 110-140, 120-140, 130-140, 100-130, 110-130, 120-130, 100-120, 110-120, or 100-110 nucleotides apart.

Chemically modified nucleic acids and nucleic acid end features

A nucleic acid described herein (e.g., a template nucleic acid, e.g., a template RNA; or a nucleic acid (e.g., mRNA) encoding a gene modifying polypeptide; or a gRNA) can comprise unmodified or modified nucleobases. Naturally occurring RNAs are synthesized from four basic ribonucleotides: ATP, CTP, UTP and GTP, but may contain post-transcriptionally modified nucleotides. Further, approximately one hundred different nucleoside modifications have been identified in RNA (Rozenski, J, Crain, P, and McCloskey, J. (1999). The RNA Modification Database: 1999 update. Nucl Acids Res 27: 196-197). An RNA can also comprise wholly synthetic nucleotides that do not occur in nature.

In some embodiments, the chemical modification is one provided in WO/2016/183482, US Pat. Pub. No. 20090286852, of International Application No. WO/2012/019168, WO/2012/045075, WO/2012/135805, WO/2012/158736, WO/2013/039857, WO/2013/039861, WO/2013/052523, WO/2013/090648, WO/2013/096709, WO/2013/101690, WO/2013/106496, WO/2013/130161, WO/2013/151669, WO/2013/151736, WO/2013/151672, WO/2013/151664, WO/2013/151665, WO/2013/151668, WO/2013/151671, WO/2013/151667, WO/2013/151670,

WO/2013/151666, WO/2013/151663, WO/2014/028429, WO/2014/081507, WO/2014/093924, WO/2014/093574, WO/2014/113089, WO/2014/144711, WO/2014/144767, WO/2014/144039, WO/2014/152540, WO/2014/152030, WO/2014/152031, WO/2014/152027, WO/2014/152211, WO/2014/158795, WO/2014/159813, WO/2014/164253, WO/2015/006747, WO/2015/034928, 5 WO/2015/034925, WO/2015/038892, WO/2015/048744, WO/2015/051214, WO/2015/051173, WO/2015/051169, WO/2015/058069, WO/2015/085318, WO/2015/089511, WO/2015/105926, WO/2015/164674, WO/2015/196130, WO/2015/196128, WO/2015/196118, WO/2016/011226, WO/2016/011222, WO/2016/011306, WO/2016/014846, WO/2016/022914, WO/2016/036902, WO/2016/077125, or WO/2016/077123, each of which is herein incorporated by reference in its 10 entirety. It is understood that incorporation of a chemically modified nucleotide into a polynucleotide can result in the modification being incorporated into a nucleobase, the backbone, or both, depending on the location of the modification in the nucleotide. In some embodiments, the backbone modification is one provided in EP 2813570, which is herein incorporated by reference in its entirety. In some embodiments, the modified cap is one provided in US Pat. Pub. 15 No. 20050287539, which is herein incorporated by reference in its entirety.

In some embodiments, the chemically modified nucleic acid (e.g., RNA, e.g., mRNA) comprises one or more of ARCA: anti-reverse cap analog (m27.3'-OGP3G), GP3G (Unmethylated Cap Analog), m7GP3G (Monomethylated Cap Analog), m32.2.7GP3G (Trimethylated Cap Analog), m5CTP (5'-methyl-cytidine triphosphate), m6ATP (N6-methyl- 20 adenosine-5'-triphosphate), s2UTP (2-thio-uridine triphosphate), and Ψ (pseudouridine triphosphate).

In some embodiments, the chemically modified nucleic acid comprises a 5' cap, e.g.: a 7-methylguanosine cap (e.g., a O-Me-m7G cap); a hypermethylated cap analog; an NAD⁺-derived cap analog (e.g., as described in Kiledjian, Trends in Cell Biology 28, 454-464 (2018)); or a 25 modified, e.g., biotinylated, cap analog (e.g., as described in Bednarek et al., Phil Trans R Soc B 373, 20180167 (2018)).

In some embodiments, the chemically modified nucleic acid comprises a 3' feature selected from one or more of: a polyA tail; a 16-nucleotide long stem-loop structure flanked by unpaired 5 nucleotides (e.g., as described by Mannironi et al., Nucleic Acid Research 17, 9113- 30 9126 (1989)); a triple-helical structure (e.g., as described by Brown et al., PNAS 109, 19202-19207 (2012)); a tRNA, Y RNA, or vault RNA structure (e.g., as described by Labno et al.,

Biochemica et Biophysica Acta 1863, 3125-3147 (2016)); incorporation of one or more deoxyribonucleotide triphosphates (dNTPs), 2'-O-Methylated NTPs, or phosphorothioate-NTPs; a single nucleotide chemical modification (e.g., oxidation of the 3' terminal ribose to a reactive aldehyde followed by conjugation of the aldehyde-reactive modified nucleotide); or chemical

5 ligation to another nucleic acid molecule.

In some embodiments, the nucleic acid (e.g., template nucleic acid) comprises one or more modified nucleotides, e.g., selected from dihydrouridine, inosine, 7-methylguanosine, 5-methylcytidine (5mC), 5' Phosphate ribothymidine, 2'-O-methyl ribothymidine, 2'-O-ethyl ribothymidine, 2'-fluoro ribothymidine, C-5 propynyl-deoxycytidine (pdC), C-5 propynyl-

10 deoxyuridine (pdU), C-5 propynyl-cytidine (pC), C-5 propynyl-uridine (pU), 5-methyl cytidine, 5-methyl uridine, 5-methyl deoxycytidine, 5-methyl deoxyuridine methoxy, 2,6-diaminopurine, 5'-Dimethoxytrityl-N4-ethyl-2'-deoxycytidine, C-5 propynyl-f-cytidine (pfC), C-5 propynyl-f-uridine (pfU), 5-methyl f-cytidine, 5-methyl f-uridine, C-5 propynyl-m-cytidine (pmC), C-5 propynyl-f-uridine (pmU), 5-methyl m-cytidine, 5-methyl m-uridine, LNA (locked nucleic acid),

15 MGB (minor groove binder) pseudouridine (Ψ), 1-N-methylpseudouridine (1-Me- Ψ), or 5-methoxyuridine (5-MO-U).

In some embodiments, the nucleic acid comprises a backbone modification, e.g., a modification to a sugar or phosphate group in the backbone. In some embodiments, the nucleic acid comprises a nucleobase modification.

20 In some embodiments, the nucleic acid comprises one or more chemically modified nucleotides of Table 9, one or more chemical backbone modifications of Table 10, one or more chemically modified caps of Table 11. For instance, in some embodiments, the nucleic acid comprises two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of chemical modifications. As an example, the nucleic acid may comprise two or more (e.g., 3, 4, 5, 6, 7, 8,

25 9, or 10 or more) different types of modified nucleobases, e.g., as described herein, e.g., in Table 9. Alternatively, or in combination, the nucleic acid may comprise two or more (e.g., 3, 4, 5, 6, 7, 8, 9, or 10 or more) different types of backbone modifications, e.g., as described herein, e.g., in Table 10. Alternatively, or in combination, the nucleic acid may comprise one or more modified cap, e.g., as described herein, e.g., in Table 11. For instance, in some embodiments,

30 the nucleic acid comprises one or more type of modified nucleobase and one or more type of backbone modification; one or more type of modified nucleobase and one or more modified cap;

one or more type of modified cap and one or more type of backbone modification; or one or more type of modified nucleobase, one or more type of backbone modification, and one or more type of modified cap.

In some embodiments, the nucleic acid comprises one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, or more) modified nucleobases. In some embodiments, all nucleobases of the nucleic acid are modified. In some embodiments, the nucleic acid is modified at one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, or more) positions in the backbone. In some embodiments, all backbone positions of the nucleic acid are modified.

Table 9. Modified nucleotides

5-aza-uridine	N2-methyl-6-thio-guanosine
2-thio-5-aza-midine	N2,N2-dimethyl-6-thio-guanosine
2-thiouridine	pyridin-4-one ribonucleoside
4-thio-pseudouridine	2-thio-5-aza-uridine
2-thio-pseudouridine	2-thiomidine
5-hydroxyuridine	4-thio-pseudomidine
3-methyluridine	2-thio-pseudowidine
5-carboxymethyl-uridine	3-methylmidine
1-carboxymethyl-pseudouridine	1-propynyl-pseudomidine
5-propynyl-uridine	1-methyl-1-deaza-pseudomidine
1-propynyl-pseudouridine	2-thio-1-methyl-1-deaza-pseudouridine
5-taurinomethyluridine	4-methoxy-pseudomidine
1-taurinomethyl-pseudouridine	5'-O-(1-Thiophosphate)-Adenosine
5-taurinomethyl-2-thio-uridine	5'-O-(1-Thiophosphate)-Cytidine
1-taurinomethyl-4-thio-uridine	5'-O-(1-thiophosphate)-Guanosine
5-methyl-uridine	5'-O-(1-Thiophosphate)-Uridine
1-methyl-pseudouridine	5'-O-(1-Thiophosphate)-Pseudouridine
4-thio-1-methyl-pseudouridine	2'-O-methyl-Adenosine
2-thio-1-methyl-pseudouridine	2'-O-methyl-Cytidine
1-methyl-1-deaza-pseudouridine	2'-O-methyl-Guanosine
2-thio-1-methyl-1-deaza-pseudomidine	2'-O-methyl-Uridine
dihydrouridine	2'-O-methyl-Pseudouridine
dihydropseudouridine	2'-O-methyl-Inosine
2-thio-dihydrouridine	2-methyladenosine
2-thio-dihydropseudouridine	2-methylthio-N6-methyladenosine
2-methoxyuridine	2-methylthio-N6 isopentenyladenosine
2-methoxy-4-thio-uridine	2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine
4-methoxy-pseudouridine	N6-methyl-N6-threonylcarbamoyladenosine
4-methoxy-2-thio-pseudouridine	N6-hydroxynorvalylcarbamoyladenosine
5-aza-cytidine	2-methylthio-N6-hydroxynorvalyl carbamoyladenosine
pseudoisocytidine	2'-O-ribosyladenosine (phosphate)
3-methyl-cytidine	1,2'-O-dimethylinosine
N4-acetylcytidine	5,2'-O-dimethylcytidine
5-formylcytidine	N4-acetyl-2'-O-methylcytidine
N4-methylcytidine	Lysidine
5-hydroxymethylcytidine	7-methylguanosine
1-methyl-pseudoisocytidine	N2,2'-O-dimethylguanosine
pyrrolo-cytidine	N2,N2,2'-O-trimethylguanosine
pyrrolo-pseudoisocytidine	2'-O-ribosylguanosine (phosphate)
2-thio-cytidine	Wybutosine
2-thio-5-methyl-cytidine	Peroxywybutosine
4-thio-pseudoisocytidine	Hydroxywybutosine
4-thio-1-methyl-pseudoisocytidine	undermodified hydroxywybutosine
4-thio-1-methyl-1-deaza-pseudoisocytidine	methylwyosine
1-methyl-1-deaza-pseudoisocytidine	queuosine

zebularine 5-aza-zebularine 5-methyl-zebularine 5-aza-2-thio-zebularine 2-thio-zebularine 2-methoxy-cytidine 2-methoxy-5-methyl-cytidine 4-methoxy-pseudoisocytidine 4-methoxy-1-methyl-pseudoisocytidine 2-aminopurine 2,6-diaminopurine 7-deaza-adenine 7-deaza-8-aza-adenine 7-deaza-2-aminopurine 7-deaza-8-aza-2-aminopurine 7-deaza-2,6-diaminopurine 7-deaza-8-aza-2,6-diaminopurine 1-methyladenosine N6-isopentenyladenosine N6-(cis-hydroxyisopentenyl)adenosine 2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine N6-glycylcarbamoyladenosine N6-threonylcarbamoyladenosine 2-methylthio-N6-threonyl carbamoyladenosine N6,N6-dimethyladenosine 7-methyladenine 2-methylthio-adenine 2-methoxy-adenine inosine 1-methyl-inosine wyosine wybutosine 7-deaza-guanosine 7-deaza-8-aza-guanosine 6-thio-guanosine 6-thio-7-deaza-guanosine 6-thio-7-deaza-8-aza-guanosine 7-methyl-guanosine 6-thio-7-methyl-guanosine 7-methylinosine 6-methoxy-guanosine 1-methylguanosine N2-methylguanosine N2,N2-dimethylguanosine 8-oxo-guanosine 7-methyl-8-oxo-guanosine 1-methyl-6-thio-guanosine	epoxyqueuosine galactosyl-queuosine mannosyl-queuosine 7-cyano-7-deazaguanosine 7-aminomethyl-7-deazaguanosine archaeosine 5,2'-O-dimethyluridine 4-thiouridine 5-methyl-2-thiouridine 2-thio-2'-O-methyluridine 3-(3-amino-3-carboxypropyl)uridine 5-methoxyuridine uridine 5-oxyacetic acid uridine 5-oxyacetic acid methyl ester 5-(carboxyhydroxymethyl)uridine 5-(carboxyhydroxymethyl)uridine methyl ester 5-methoxycarbonylmethyluridine 5-methoxycarbonylmethyl-2'-O-methyluridine 5-methoxycarbonylmethyl-2-thiouridine 5-aminomethyl-2-thiouridine 5-methylaminomethyluridine 5-methylaminomethyl-2-thiouridine 5-methylaminomethyl-2-selenouridine 5-carbamoylmethyluridine 5-carbamoylmethyl-2'-O-methyluridine 5-carboxymethylaminomethyluridine 5-carboxymethylaminomethyl-2'-O-methyluridine 5-carboxymethylaminomethyl-2-thiouridine N4,2'-O-dimethylcytidine 5-carboxymethyluridine N6,2'-O-dimethyladenosine N,N6,O-2'-trimethyladenosine N2,7-dimethylguanosine N2,N2,7-trimethylguanosine 3,2'-O-dimethyluridine 5-methyldihydrouridine 5-formyl-2'-O-methylcytidine 1,2'-O-dimethylguanosine 4-demethylwyosine Isowyosine N6-acetyladenosine
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Table 10. Backbone modifications

2'-O-Methyl backbone Peptide Nucleic Acid (PNA) backbone phosphorothioate backbone morpholino backbone carbamate backbone siloxane backbone sulfide backbone sulfoxide backbone sulfone backbone formacetyl backbone thioformacetyl backbone methyleneformacetyl backbone riboacetyl backbone alkene containing backbone sulfamate backbone sulfonate backbone sulfonamide backbone methyleneimino backbone
--

methylenedihydrazino backbone amide backbone

Table 11. Modified caps

m7GpppA m7GpppC m2,7GpppG m2,2,7GpppG m7Gpppm7G m7,2'OmeGpppG m7,2'dGpppG m7,3'OmeGpppG m7,3'dGpppG GppppG m7GppppG m7GppppA m7GppppC m2,7GppppG m2,2,7GppppG m7Gppppm7G m7,2'OmeGppppG m7,2'dGppppG m7,3'OmeGppppG m7,3'dGppppG

The nucleotides comprising the template of the gene modifying system can be natural or modified bases, or a combination thereof. For example, the template may contain pseudouridine, dihydrouridine, inosine, 7-methylguanosine, or other modified bases. In some embodiments, the template may contain locked nucleic acid nucleotides. In some embodiments, the modified bases used in the template do not inhibit the reverse transcription of the template. In some embodiments, the modified bases used in the template may improve reverse transcription, e.g., specificity or fidelity.

In some embodiments, an RNA component of the system (e.g., a template RNA or a gRNA) comprises one or more nucleotide modifications. In some embodiments, the modification pattern of a gRNA can significantly affect *in vivo* activity compared to unmodified or end-modified guides (e.g., as shown in Figure 1D from Finn et al. *Cell Rep* 22(9):2227-2235 (2018); incorporated herein by reference in its entirety). Without wishing to be bound by theory, this process may be due, at least in part, to a stabilization of the RNA conferred by the modifications. Non-limiting examples of such modifications may include 2'-O-methyl (2'-O-Me), 2'-O-(2-methoxyethyl) (2'-O-MOE), 2'-fluoro (2'-F), phosphorothioate (PS) bond between nucleotides, G-C substitutions, and inverted abasic linkages between nucleotides and equivalents thereof.

In some embodiments, the template RNA (e.g., at the portion thereof that binds a target site) or the guide RNA comprises a 5' terminus region. In some embodiments, the template RNA or the guide RNA does not comprise a 5' terminus region. In some embodiments, the 5' terminus region comprises a gRNA spacer region, e.g., as described with respect to sgRNA in Briner AE et al, Molecular Cell 56: 333-339 (2014) (incorporated herein by reference in its entirety; applicable herein, e.g., to all guide RNAs). In some embodiments, the 5' terminus region comprises a 5' end modification. In some embodiments, a 5' terminus region with or without a spacer region may be associated with a crRNA, trRNA, sgRNA and/or dgRNA. The gRNA spacer region can, in some instances, comprise a guide region, guide domain, or targeting domain.

In some embodiments, the template RNAs (e.g., at the portion thereof that binds a target site) or guide RNAs described herein comprises any of the sequences shown in Table 4 of WO2018107028A1, incorporated herein by reference in its entirety. In some embodiments, where a sequence shows a guide and/or spacer region, the composition may comprise this region or not. In some embodiments, a guide RNA comprises one or more of the modifications of any of the sequences shown in Table 4 of WO2018107028A1, e.g., as identified therein by a SEQ ID NO. In embodiments, the nucleotides may be the same or different, and/or the modification pattern shown may be the same or similar to a modification pattern of a guide sequence as shown in Table 4 of WO2018107028A1. In some embodiments, a modification pattern includes the relative position and identity of modifications of the gRNA or a region of the gRNA (e.g. 5' terminus region, lower stem region, bulge region, upper stem region, nexus region, hairpin 1 region, hairpin 2 region, 3' terminus region). In some embodiments, the modification pattern contains at least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the modifications of any one of the sequences shown in the sequence column of Table 4 of WO2018107028A1, and/or over one or more regions of the sequence. In some embodiments, the modification pattern is at least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the modification pattern of any one of the sequences shown in the sequence column of Table 4 of WO2018107028A1. In some embodiments, the modification pattern is at least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over one or more regions of the sequence shown in Table 4 of WO2018107028A1, e.g., in a 5' terminus region, lower stem region, bulge region, upper stem

region, nexus region, hairpin 1 region, hairpin 2 region, and/or 3' terminus region. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the modification pattern of a sequence over the 5' terminus region. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the lower stem. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the bulge. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the upper stem. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the nexus. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the hairpin 1. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the hairpin 2. In some embodiments, the modification pattern is least 50%, 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical over the 3' terminus. In some embodiments, the modification pattern differs from the modification pattern of a sequence of Table 4 of WO2018107028A1, or a region (e.g. 5' terminus, lower stem, bulge, upper stem, nexus, hairpin 1, hairpin 2, 3' terminus) of such a sequence, e.g., at 0, 1, 2, 3, 4, 5, 6, or more nucleotides. In some embodiments, the gRNA comprises modifications that differ from the modifications of a sequence of Table 4 of WO2018107028A1, e.g., at 0, 1, 2, 3, 4, 5, 6, or more nucleotides. In some embodiments, the gRNA comprises modifications that differ from modifications of a region (e.g. 5' terminus, lower stem, bulge, upper stem, nexus, hairpin 1, hairpin 2, 3' terminus) of a sequence of Table 4 of WO2018107028A1, e.g., at 0, 1, 2, 3, 4, 5, 6, or more nucleotides.

25 In some embodiments, the template RNAs (e.g., at the portion thereof that binds a target site) or the gRNA comprises a 2'-O-methyl (2'-O-Me) modified nucleotide. In some embodiments, the gRNA comprises a 2'-O-(2-methoxy ethyl) (2'-O-moe) modified nucleotide. In some embodiments, the gRNA comprises a 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the gRNA comprises a phosphorothioate (PS) bond between nucleotides. In some

30 end modifications. In some embodiments, the 5' end modification comprises a phosphorothioate

(PS) bond between nucleotides. In some embodiments, the 5' end modification comprises a 2'-O-methyl (2'-O-Me), 2'-O-(2-methoxy ethyl) (2'-O-MOE), and/or 2'-fluoro (2'-F) modified nucleotide. In some embodiments, the 5' end modification comprises at least one phosphorothioate (PS) bond and one or more of a 2'-O-methyl (2'-O-Me), 2'-O-(2-methoxyethyl) (2'-O-MOE), and/or 2'-fluoro (2'-F) modified nucleotide. The end modification may comprise a phosphorothioate (PS), 2'-O-methyl (2'-O-Me), 2'-O-(2-methoxyethyl) (2'-O-MOE), and/or 2'-fluoro (2'-F) modification. Equivalent end modifications are also encompassed by embodiments described herein. In some embodiments, the template RNA or gRNA comprises an end modification in combination with a modification of one or more regions of the template RNA or gRNA. Additional exemplary modifications and methods for protecting RNA, e.g., gRNA, and formulae thereof, are described in WO2018126176A1, which is incorporated herein by reference in its entirety.

In some embodiments, structure-guided and systematic approaches are used to introduce modifications (e.g., 2'-OMe-RNA, 2'-F-RNA, and PS modifications) to a template RNA or guide RNA, for example, as described in Mir et al. *Nat Commun* 9:2641 (2018) (incorporated by reference herein in its entirety). In some embodiments, the incorporation of 2'-F-RNAs increases thermal and nuclease stability of RNA:RNA or RNA:DNA duplexes, e.g., while minimally interfering with C3'-endo sugar pucker. In some embodiments, 2'-F may be better tolerated than 2'-OMe at positions where the 2'-OH is important for RNA:DNA duplex stability. In some embodiments, a crRNA comprises one or more modifications that do not reduce Cas9 activity, e.g., C10, C20, or C21 (fully modified), e.g., as described in Supplementary Table 1 of Mir et al. *Nat Commun* 9:2641 (2018), incorporated herein by reference in its entirety. In some embodiments, a tracrRNA comprises one or more modifications that do not reduce Cas9 activity, e.g., T2, T6, T7, or T8 (fully modified) of Supplementary Table 1 of Mir et al. *Nat Commun* 9:2641 (2018). In some embodiments, a crRNA comprises one or more modifications (e.g., as described herein) may be paired with a tracrRNA comprising one or more modifications, e.g., C20 and T2. In some embodiments, a gRNA comprises a chimera, e.g., of a crRNA and a tracrRNA (e.g., Jinek et al. *Science* 337(6096):816-821 (2012)). In embodiments, modifications from the crRNA and tracrRNA are mapped onto the single-guide chimera, e.g., to produce a modified gRNA with enhanced stability.

In some embodiments, gRNA molecules may be modified by the addition or subtraction of the naturally occurring structural components, e.g., hairpins. In some embodiments, a gRNA may comprise a gRNA with one or more 3' hairpin elements deleted, e.g., as described in WO2018106727, incorporated herein by reference in its entirety. In some embodiments, a
5 gRNA may contain an added hairpin structure, e.g., an added hairpin structure in the spacer region, which was shown to increase specificity of a CRISPR-Cas system in the teachings of Kocak et al. *Nat Biotechnol* 37(6):657-666 (2019). Additional modifications, including examples of shortened gRNA and specific modifications improving *in vivo* activity, can be found in US20190316121, incorporated herein by reference in its entirety.

10 In some embodiments, structure-guided and systematic approaches (e.g., as described in Mir et al. *Nat Commun* 9:2641 (2018); incorporated herein by reference in its entirety) are employed to find modifications for the template RNA. In embodiments, the modifications are identified with the inclusion or exclusion of a guide region of the template RNA. In some
15 embodiments, a structure of polypeptide bound to template RNA is used to determine non-protein-contacted nucleotides of the RNA that may then be selected for modifications, e.g., with lower risk of disrupting the association of the RNA with the polypeptide. Secondary structures in a template RNA can also be predicted *in silico* by software tools, e.g., the RNAstructure tool available at rna.urmc.rochester.edu/RNAstructureWeb (Bellaousov et al. *Nucleic Acids Res*
41:W471-W474 (2013); incorporated by reference herein in its entirety), e.g., to determine
20 secondary structures for selecting modifications, e.g., hairpins, stems, and/or bulges.

Production of Compositions and Systems

As will be appreciated by one of skill, methods of designing and constructing nucleic acid constructs and proteins or polypeptides (such as the systems, constructs and polypeptides described herein) are routine in the art. Generally, recombinant methods may be used. See, in
25 general, Smales & James (Eds.), *Therapeutic Proteins: Methods and Protocols* (Methods in Molecular Biology), Humana Press (2005); and Crommelin, Sindelar & Meibohm (Eds.), *Pharmaceutical Biotechnology: Fundamentals and Applications*, Springer (2013). Methods of designing, preparing, evaluating, purifying and manipulating nucleic acid compositions are described in Green and Sambrook (Eds.), *Molecular Cloning: A Laboratory Manual* (Fourth
30 Edition), Cold Spring Harbor Laboratory Press (2012).

The disclosure provides, in part, a nucleic acid, e.g., vector, encoding a gene modifying polypeptide described herein, a template nucleic acid described herein, or both. In some embodiments, a vector comprises a selective marker, e.g., an antibiotic resistance marker. In some embodiments, the antibiotic resistance marker is a kanamycin resistance marker. In some
5 embodiments, the antibiotic resistance marker does not confer resistance to beta-lactam antibiotics. In some embodiments, the vector does not comprise an ampicillin resistance marker. In some embodiments, the vector comprises a kanamycin resistance marker and does not comprise an ampicillin resistance marker. In some embodiments, a vector encoding a gene modifying polypeptide is integrated into a target cell genome (e.g., upon administration to a
10 target cell, tissue, organ, or subject). In some embodiments, a vector encoding a gene modifying polypeptide is not integrated into a target cell genome (e.g., upon administration to a target cell, tissue, organ, or subject). In some embodiments, a vector encoding a template nucleic acid (e.g., template RNA) is not integrated into a target cell genome (e.g., upon administration to a target cell, tissue, organ, or subject). In some embodiments, if a vector is integrated into a target site in
15 a target cell genome, the selective marker is not integrated into the genome. In some embodiments, if a vector is integrated into a target site in a target cell genome, genes or sequences involved in vector maintenance (e.g., plasmid maintenance genes) are not integrated into the genome. In some embodiments, if a vector is integrated into a target site in a target cell genome, transfer regulating sequences (e.g., inverted terminal repeats, e.g., from an AAV) are
20 not integrated into the genome. In some embodiments, administration of a vector (e.g., encoding a gene modifying polypeptide described herein, a template nucleic acid described herein, or both) to a target cell, tissue, organ, or subject results in integration of a portion of the vector into one or more target sites in the genome(s) of said target cell, tissue, organ, or subject. In some
embodiments, less than 99, 95, 90, 80, 70, 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, or 1% of target sites
25 (e.g., no target sites) comprising integrated material comprise a selective marker (e.g., an antibiotic resistance gene), a transfer regulating sequence (e.g., an inverted terminal repeat, e.g., from an AAV), or both from the vector.

Exemplary methods for producing a therapeutic pharmaceutical protein or polypeptide described herein involve expression in mammalian cells, although recombinant proteins can also
30 be produced using insect cells, yeast, bacteria, or other cells under control of appropriate promoters. Mammalian expression vectors may comprise non-transcribed elements such as an

origin of replication, a suitable promoter, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and termination sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, splice, and polyadenylation sites may be used to provide other genetic elements required for expression of a heterologous DNA sequence. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described in Green & Sambrook, *Molecular Cloning: A Laboratory Manual* (Fourth Edition), Cold Spring Harbor Laboratory Press (2012).

Various mammalian cell culture systems can be employed to express and manufacture recombinant protein. Examples of mammalian expression systems include CHO, COS, HEK293, HeLA, and BHK cell lines. Processes of host cell culture for production of protein therapeutics are described in Zhou and Kantardjieff (Eds.), *Mammalian Cell Cultures for Biologics Manufacturing (Advances in Biochemical Engineering/Biotechnology)*, Springer (2014). Compositions described herein may include a vector, such as a viral vector, e.g., a lentiviral vector, encoding a recombinant protein. In some embodiments, a vector, e.g., a viral vector, may comprise a nucleic acid encoding a recombinant protein.

Purification of protein therapeutics is described in Franks, *Protein Biotechnology: Isolation, Characterization, and Stabilization*, Humana Press (2013); and in Cutler, *Protein Purification Protocols (Methods in Molecular Biology)*, Humana Press (2010).

The disclosure also provides compositions and methods for the production of template nucleic acid molecules (e.g., template RNAs) with specificity for a gene modifying polypeptide and/or a genomic target site. In an aspect, the method comprises production of RNA segments including an upstream homology segment, a heterologous object sequence segment, a gene modifying polypeptide binding motif, and a gRNA segment.

Applications

Therapeutic Applications

In some embodiments, a gene modifying system as described herein can be used to modify a cell (e.g., an animal cell, plant cell, or fungal cell). In some embodiments, a gene modifying system as described herein can be used to modify a mammalian cell (e.g., a human

cell). In some embodiments, a gene modifying system as described herein can be used to modify a cell from a livestock animal (e.g., a cow, horse, sheep, goat, pig, llama, alpaca, camel, yak, chicken, duck, goose, or ostrich). In some embodiments, a gene modifying system as described herein can be used as a laboratory tool or a research tool, or used in a laboratory method or
5 research method, e.g., to modify an animal cell, e.g., a mammalian cell (e.g., a human cell), a plant cell, or a fungal cell.

By integrating coding genes into a RNA sequence template, the gene modifying system can address therapeutic needs, for example, by providing expression of a therapeutic transgene in individuals with loss-of-function mutations, by replacing gain-of-function mutations with normal
10 transgenes, by providing regulatory sequences to eliminate gain-of-function mutation expression, and/or by controlling the expression of operably linked genes, transgenes and systems thereof. In certain embodiments, the RNA sequence template encodes a promotor region specific to the therapeutic needs of the host cell, for example a tissue specific promotor or enhancer. In still other embodiments, a promotor can be operably linked to a coding sequence.

15 In some embodiments, an insertion, deletion, substitution, or combination thereof, increases or decreases expression (e.g. transcription or translation) of a target gene. In some embodiments, an insertion, deletion, substitution, or combination thereof, increases or decreases expression (e.g. transcription or translation) of a target gene by altering, adding, or deleting sequences in a promoter or enhancer, e.g. sequences that bind transcription factors. In some
20 embodiments, an insertion, deletion, substitution, or combination thereof alters translation of a target gene (e.g. alters an amino acid sequence), inserts or deletes a start or stop codon, alters or fixes the translation frame of a gene. In some embodiments, an insertion, deletion, substitution, or combination thereof alters splicing of a target gene, e.g. by inserting, deleting, or altering a splice acceptor or donor site. In some embodiments, an insertion, deletion, substitution, or
25 combination thereof alters transcript or protein half-life. In some embodiments, an insertion, deletion, substitution, or combination thereof, alters, increases, decreases the activity of a target gene, e.g. a protein encoded by the target gene.

Compensatory edits

In some embodiments, the systems or methods provided herein can be used to introduce a
30 compensatory edit. In some embodiments, the compensatory edit is at a position of a gene associated with a disease or disorder, which is different from the position of a disease-causing

mutation. In some embodiments, the compensatory mutation is not in the gene containing the causative mutation. In some embodiments, the compensatory edit can negate or compensate for a disease-causing mutation. In some embodiments, the compensatory edit can be introduced by the systems or methods provided herein to suppress or reverse the mutant effect of a disease-causing mutation.

Regulatory edits

In some embodiments, the systems or methods provided herein can be used to introduce a regulatory edit. In some embodiments, the regulatory edit is introduced to a regulatory sequence of a gene, for example, a gene promoter, gene enhancer, gene repressor, or a sequence that regulates gene splicing. In some embodiments, the regulatory edit increases or decreases the expression level of a target gene. In some embodiments, the target gene is the same as the gene containing a disease-causing mutation. In some embodiments, the target gene is different from the gene containing a disease-causing mutation.

Repeat expansion diseases

In some embodiments, the systems or methods provided herein can be used to treat a repeat expansion disease. In some embodiments, the systems or methods provided herein, for example, those comprising gene modifying polypeptides, can be used to treat repeat expansion diseases by resetting the number of repeats at the locus according to a customized RNA template.

Therapeutic Indications

In some embodiments the systems or methods provided herein can be used to treat an indication of any of Tables 12-15, below. For instance, in some embodiments the gene modifying system modifies a target site in genomic DNA in a cell, wherein the target site is in a gene of any of Tables 12-15, e.g., in a subject having the corresponding indication listed in any of Tables 12-15. In some embodiments, the cell is a liver cell, and the target site is in a gene of Table 12, e.g., in a subject having the corresponding indication listed in Table 12. In some embodiments, the cell is a hematopoietic stem cell (HSC), and the target site is in a gene of Table 13, e.g., in a subject having the corresponding indication listed in Table 13. In some embodiments, the cell is a central nervous system (CNS) cell, and the target site is in a gene of Table 14, e.g., in a subject having the corresponding indication listed in Table 14. In some embodiments, the cell is a cell of the eye, and the target site is in a gene of Table 15, e.g., in a subject having the corresponding indication listed in Table 15. In some embodiments, the target

site is in a coding region in the gene. In some embodiments, the target site is in a promoter. In some embodiments, the target site is in a 5' UTR or a 3' UTR of the gene of any of Tables 12-15. In some embodiments, the target site is in an intron or exon of the gene. In some embodiments, the gene modifying system corrects a mutation in the gene. In some embodiments, the gene modifying polypeptide inserts a sequence that had been deleted from the gene (e.g., through a disease-causing mutation). In some embodiments, the gene modifying system deletes a sequence that had been duplicated in the gene (e.g., through a disease-causing mutation). In some embodiments, the gene modifying system replaces a mutation (e.g., a disease-causing mutation) with the corresponding wild-type sequence. In some embodiments, the mutation is a substitution, insertion, deletion, or inversion.

Table 12: Indications and genetic targets, e.g., in the liver

Disease	Gene Affected
Acute intermittent porphyria	HMBS
Alpha-1-antitrypsin deficiency (AAT)	SERPINA1
Arginase deficiency	ARG1
Argininosuccinate lyase deficiency	ASL
Carbamoyl phosphate synthetase I deficiency	CPS1
Citrin deficiency	SLC25A13
Citrullinemia type I	ASS1
Crigler-Najjar syndrome (Hyperbilirubinemia)	UGT1A1
Fabry disease	GLA
Familial hypercholesterolemia 4 (homozygous familial cholesterolemia)	LDLRAP1
Glutaric aciduria I	GCDH
Glutaric aciduria II (multiple acyl-CoA dehydrogenase deficiency)	GA IIA: ETFA GA IIB: ETFB GA IIC: ETFDH
Glycogen storage disease type IV	GBE1
Hemophilia A	F8
Hemophilia B	F9
Hereditary hemochromatosis	HFE
Homocystinuria	CBS
Maple syrup urine disease (MSUD)	Type Ia: BCKDHA Type Ib: BCKDHB Type II: DBT
Methylmalonic acidemia (methylmalonyl-CoA mutase deficiency)	MMUT
MPS 1S (Scheie syndrome)	IDUA
MPS2	IDS
MPS3 (San Filippo Syndrome)	Type IIIa: SGSH Type IIIb: NAGLU Type IIIc: HGSNAT Type IIId: GNS
MPS4	Type IVA: GALNS Type IVB: GLB1

Disease	Gene Affected
MPS6	ARSB
MPS7	GUSB
Ornithine transcarbamylase deficiency	OTC
Phenylketonuria (phenylalanine hydroxylase deficiency)	PAH
Polycystic Liver Disease	PCLD1: PRKCSH PCLD2: SEC63 PCLD3: ALG8 PCLD4: LRP5
Pompe disease	GAA
Primary Hyperoxaluria 1 (oxalosis)	AGXT
Progressive familial intrahepatic cholestasis type 1	ATP8B1
Progressive familial intrahepatic cholestasis type 2	ABCB11
Progressive familial intrahepatic cholestasis type 3	ABCB4
Propionic acidemia	PCCB; PCCA
Pyruvate carboxylase deficiency	PC
Tyrosinemia type I	FAH
Wilson's disease	ATP7B

Table 13: Indications and genetic targets for HSCs

Disease	Gene Affected
Adrenoleukodystrophy (CALD)	ABCD1
Alpha-mannosidosis	MAN2B1
Blackfan-Diamond Anemia	
Congenital amegakaryocytic thrombocytopenia	MPL
Dyskeratosis Congenita	TERC
Fanconi anemia	FANC
Gaucher disease	GBA
Globoid cell leukodystrophy (Krabbe disease)	GALC
Hemophagocytic lymphohistiocytosis	PRF1; STX11; STXBP2; UNC13D
Malignant infantile osteopetrosis- autosomal recessive osteopetrosis	Many genes implicated
Metachromatic leukodystrophy	PSAP
MPS 1S (Scheie syndrome)	IDUA
MPS2	IDS
MPS7	GUSB
Mucopolipidosis II	GNPTAB
Niemann-Pick disease A and B	SMPD1
Niemann-Pick disease C	NPC1
Pompe disease	GAA
Pyruvate kinase deficiency (PKD)	PKLR
Sickle cell disease (SCD)	HBB
Tay Sachs	HEXA
Thalassemia	HBB

Table 14: Indications and genetic targets for the CNS

Disease	Gene Affected
Alpha-mannosidosis	MAN2B1
Ataxia-telangiectasia	ATM
CADASIL	NOTCH3
Canavan disease	ASPA
Carbamoyl-phosphate synthetase 1 deficiency	CPS1
CLN1 disease	PPT1
CLN2 Disease	TPP1
CLN3 Disease (Juvenile neuronal ceroid lipofuscinosis, Batten Disease)	CLN3
Coffin-Lowry syndrome	RPS6KA3
Congenital myasthenic syndrome 5	COLQ
Cornelia de Lange syndrome (NIPBL)	NIPBL
Cornelia de Lange syndrome (SMC1A)	SMC1A
Dravet syndrome (SCN1A)	SCN1A
Glycine encephalopathy (GLDC)	GLDC
GM1 gangliosidosis	GLB1
Huntington's Disease	HTT
Hydrocephalus with stenosis of the aqueduct of Sylvius	L1CAM
Leigh Syndrome	SURF1
Metachromatic leukodystrophy (ARSA)	ARSA
MPS type 2	IDS
MPS type 3	Type 3a: SGSH Type 3b: NAGLU
Mucopolidosis IV	MCOLN1
Neurofibromatosis Type 1	NF1
Neurofibromatosis type 2	NF2
Pantothenate kinase-associated neurodegeneration	PANK2
Pyridoxine-dependent epilepsy	ALDH7A1
Rett syndrome (MECP2)	MECP2
Sandhoff disease	HEXB
Semantic dementia (Frontotemporal dementia)	MAPT
Spinocerebellar ataxia with axonal neuropathy (Ataxia with Oculomotor Apraxia)	SETX
Tay-Sachs disease	HEXA
X-linked Adrenoleukodystrophy	ABCD1

Table 15: Indications and genetic targets for the eye

Disease	Gene Affected
Achromatopsia	CNGB3
Amaurosis Congenita (LCA1)	GUCY2D
Amaurosis Congenita (LCA10)	CEP290
Amaurosis Congenita (LCA2)	RPE65
Amaurosis Congenita (LCA8)	CRB1

Disease	Gene Affected
Choroideremia	CHM
Cone Rod Dystrophy (ABCA4)	ABCA4
Cone Rod Dystrophy (GUCY2D)	GUCY2D
Cystinosis, Ocular Nonnephropathic	CTNS
Doyme Honeycomb Retinal Dystrophy (DHRD)	EFEMP1
Familial Oculoleptomeningeal Amyloidosis	TTR
Keratitis-ichthyosis-deafness (KID)	GJB2
Lattice corneal dystrophy type I	TGFBI
Macular Corneal Dystrophy (MCD)	CHST6
Meesmann Corneal Dystrophy	KRT12; KRT3
Optic Atrophy	OPA1
Retinitis Pigmentosa (AR)	USH2A
Retinitis Pigmentosa (AD)	RHO
Sorsby Fundus Dystrophy	TIMP3
Stargardt Disease	ABCA4

Application to Plants

In some embodiments, the systems or methods provided herein can be used to modify a plant or a plant part (e.g., leaves, roots, flowers, fruits, or seeds), e.g., to increase the fitness of a plant.

Delivery to a Plant

Provided herein are methods of delivering a gene modifying system described herein to a plant. Included are methods for delivering a gene modifying system to a plant by contacting the plant, or part thereof, with a gene modifying system. The methods are useful for modifying the plant to, e.g., increase the fitness of a plant.

More specifically, in some embodiments, a nucleic acid described herein (e.g., a nucleic acid encoding a gene modifying system) may be encoded in a vector, e.g., inserted adjacent to a plant promoter, e.g., a maize ubiquitin promoter (ZmUBI) in a plant vector (e.g., pHUC411). In some embodiments, the nucleic acids described herein are introduced into a plant (e.g., japonica rice) or part of a plant (e.g., a callus of a plant) via agrobacteria. In some embodiments, the systems and methods described herein can be used in plants by replacing a plant gene (e.g., hygromycin phosphotransferase (HPT)) with a null allele (e.g., containing a base substitution at the start codon). Systems and methods for modifying a plant genome are described in Xu et. al.

Development of plant prime-editing systems for precise genome editing, 2020, Plant Communications.

In one aspect, provided herein is a method of increasing the fitness of a plant, the method including delivering to the plant the gene modifying system described herein (e.g., in an effective
5 amount and duration) to increase the fitness of the plant relative to an untreated plant (e.g., a plant that has not been delivered the gene modifying system).

An increase in the fitness of the plant as a consequence of delivery of a gene modifying system can manifest in a number of ways, e.g., thereby resulting in a better production of the plant, for example, an improved yield, improved vigor of the plant or quality of the harvested
10 product from the plant, an improvement in pre- or post-harvest traits deemed desirable for agriculture or horticulture (e.g., taste, appearance, shelf life), or for an improvement of traits that otherwise benefit humans (e.g., decreased allergen production). An improved yield of a plant relates to an increase in the yield of a product (e.g., as measured by plant biomass, grain, seed or fruit yield, protein content, carbohydrate or oil content or leaf area) of the plant by a measurable
15 amount over the yield of the same product of the plant produced under the same conditions, but without the application of the instant compositions or compared with application of conventional plant-modifying agents. For example, yield can be increased by at least about 0.5%, about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 100%, or more than 100%. In some
20 instances, the method is effective to increase yield by about 2x-fold, 5x-fold, 10x-fold, 25x-fold, 50x-fold, 75x-fold, 100x-fold, or more than 100x-fold relative to an untreated plant. Yield can be expressed in terms of an amount by weight or volume of the plant or a product of the plant on some basis. The basis can be expressed in terms of time, growing area, weight of plants produced, or amount of a raw material used. For example, such methods may increase the yield
25 of plant tissues including, but not limited to: seeds, fruits, kernels, bolls, tubers, roots, and leaves.

An increase in the fitness of a plant as a consequence of delivery of a gene modifying system can also be measured by other means, such as an increase or improvement of the vigor rating, the stand (the number of plants per unit of area), plant height, stalk circumference, stalk length, leaf number, leaf size, plant canopy, visual appearance (such as greener leaf color), root
30 rating, emergence, protein content, increased tillering, bigger leaves, more leaves, fewer dead

basal leaves, stronger tillers, less fertilizer needed, fewer seeds needed, more productive tillers, earlier flowering, early grain or seed maturity, less plant verse (lodging), increased shoot growth, earlier germination, or any combination of these factors, by a measurable or noticeable amount over the same factor of the plant produced under the same conditions, but without the
5 administration of the instant compositions or with application of conventional plant-modifying agents.

Accordingly, provided herein is a method of modifying a plant, the method including delivering to the plant an effective amount of any of the gene modifying systems provided herein, wherein the method modifies the plant and thereby introduces or increases a beneficial
10 trait in the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant. In particular, the method may increase the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In some instances, the increase in plant fitness is an increase (e.g., by about 1%, 2%, 5%,
15 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in disease resistance, drought tolerance, heat tolerance, cold tolerance, salt tolerance, metal tolerance, herbicide tolerance, chemical tolerance, water use efficiency, nitrogen utilization, resistance to nitrogen stress, nitrogen fixation, pest resistance, herbivore resistance, pathogen resistance, yield, yield under water-limited conditions, vigor, growth, photosynthetic capability, nutrition, protein
20 content, carbohydrate content, oil content, biomass, shoot length, root length, root architecture, seed weight, or amount of harvestable produce.

In some instances, the increase in fitness is an increase (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in development, growth, yield, resistance to abiotic stressors, or resistance to biotic stressors. An abiotic stress
25 refers to an environmental stress condition that a plant or a plant part is subjected to that includes, e.g., drought stress, salt stress, heat stress, cold stress, and low nutrient stress. A biotic stress refers to an environmental stress condition that a plant or plant part is subjected to that includes, e.g. nematode stress, insect herbivory stress, fungal pathogen stress, bacterial pathogen stress, or viral pathogen stress. The stress may be temporary, e.g. several hours, several days,
30 several months, or permanent, e.g. for the life of the plant.

In some instances, the increase in plant fitness is an increase (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in quality of products harvested from the plant. For example, the increase in plant fitness may be an improvement in commercially favorable features (e.g., taste or appearance) of a product
5 harvested from the plant. In other instances, the increase in plant fitness is an increase in shelf-life of a product harvested from the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%).

Alternatively, the increase in fitness may be an alteration of a trait that is beneficial to human or animal health, such as a reduction in allergen production. For example, the increase in
10 fitness may be a decrease (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) in production of an allergen (e.g., pollen) that stimulates an immune response in an animal (e.g., human).

The modification of the plant (e.g., increase in fitness) may arise from modification of one or more plant parts. For example, the plant can be modified by contacting leaf, seed, pollen, root, fruit, shoot, flower, cells, protoplasts, or tissue (e.g., meristematic tissue) of the plant. As
15 such, in another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting pollen of the plant with an effective amount of any of the plant-modifying compositions herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than
20 100%) relative to an untreated plant.

In yet another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting a seed of the plant with an effective amount of any of the gene modifying systems disclosed herein, wherein the method increases the fitness of the plant (e.g.,
by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than
25 100%) relative to an untreated plant.

In another aspect, provided herein is a method including contacting a protoplast of the plant with an effective amount of any of the gene modifying systems described herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than 100%) relative to an untreated plant.

In a further aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting a plant cell of the plant with an effective amount of any of the gene modifying system described herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than
5 100%) relative to an untreated plant.

In another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting meristematic tissue of the plant with an effective amount of any of the plant-modifying compositions herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more
10 than 100%) relative to an untreated plant.

In another aspect, provided herein is a method of increasing the fitness of a plant, the method including contacting an embryo of the plant with an effective amount of any of the plant-modifying compositions herein, wherein the method increases the fitness of the plant (e.g., by about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more than
15 100%) relative to an untreated plant.

Plant Administration Methods

A plant described herein can be exposed to any of the gene modifying system compositions described herein in any suitable manner that permits delivering or administering the composition to the plant. The gene modifying system may be delivered either alone or in
20 combination with other active (e.g., fertilizing agents) or inactive substances and may be applied by, for example, spraying, injection (e.g., microinjection), through plants, pouring, dipping, in the form of concentrated liquids, gels, solutions, suspensions, sprays, powders, pellets, briquettes, bricks and the like, formulated to deliver an effective concentration of the plant-modifying composition. Amounts and locations for application of the compositions described
25 herein are generally determined by the habitat of the plant, the lifecycle stage at which the plant can be targeted by the plant-modifying composition, the site where the application is to be made, and the physical and functional characteristics of the plant-modifying composition.

In some instances, the composition is sprayed directly onto a plant, e.g., crops, by e.g., backpack spraying, aerial spraying, crop spraying/dusting etc. In instances where the gene

modifying system is delivered to a plant, the plant receiving the gene modifying system may be at any stage of plant growth. For example, formulated plant-modifying compositions can be applied as a seed-coating or root treatment in early stages of plant growth or as a total plant treatment at later stages of the crop cycle. In some instances, the plant-modifying composition
5 may be applied as a topical agent to a plant.

Further, the gene modifying system may be applied (e.g., in the soil in which a plant grows, or in the water that is used to water the plant) as a systemic agent that is absorbed and distributed through the tissues of a plant. In some instances, plants or food organisms may be genetically transformed to express the gene modifying system.

10 Delayed or continuous release can also be accomplished by coating the gene modifying system or a composition with the plant-modifying composition(s) with a dissolvable or bioerodable coating layer, such as gelatin, which coating dissolves or erodes in the environment of use, to then make the plant-modifying system position available, or by dispersing the agent in a dissolvable or erodable matrix. Such continuous release and/or dispensing means devices may
15 be advantageously employed to consistently maintain an effective concentration of one or more of the plant-modifying compositions described herein.

In some instances, the gene modifying system is delivered to a part of the plant, e.g., a leaf, seed, pollen, root, fruit, shoot, or flower, or a tissue, cell, or protoplast thereof. In some instances, the gene modifying system is delivered to a cell of the plant. In some instances, the
20 gene modifying system is delivered to a protoplast of the plant. In some instances, the gene modifying system is delivered to a tissue of the plant. For example, the composition may be delivered to meristematic tissue of the plant (e.g., apical meristem, lateral meristem, or intercalary meristem). In some instances, the composition is delivered to permanent tissue of the plant (e.g., simple tissues (e.g., parenchyma, collenchyma, or sclerenchyma) or complex
25 permanent tissue (e.g., xylem or phloem)). In some instances, the gene modifying system is delivered to a plant embryo.

Plants

A variety of plants can be delivered to or treated with a gene modifying system described herein. Plants that can be delivered a gene modifying system (i.e., “treated”) in accordance with
30 the present methods include whole plants and parts thereof, including, but not limited to, shoot

vegetative organs/structures (e.g., leaves, stems and tubers), roots, flowers and floral organs/structures (e.g., bracts, sepals, petals, stamens, carpels, anthers and ovules), seed (including embryo, endosperm, cotyledons, and seed coat) and fruit (the mature ovary), plant tissue (e.g., vascular tissue, ground tissue, and the like) and cells (e.g., guard cells, egg cells, and the like), and progeny of same. Plant parts can further refer to parts of the plant such as the shoot, root, stem, seeds, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, and the like.

The class of plants that can be treated in a method disclosed herein includes the class of higher and lower plants, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, horsetails, psilophytes, lycophytes, bryophytes, and algae (e.g., multicellular or unicellular algae). Plants that can be treated in accordance with the present methods further include any vascular plant, for example monocotyledons or dicotyledons or gymnosperms, including, but not limited to alfalfa, apple, Arabidopsis, banana, barley, canola, castor bean, chrysanthemum, clover, cocoa, coffee, cotton, cottonseed, corn, crambe, cranberry, cucumber, dendrobium, dioscorea, eucalyptus, fescue, flax, gladiolus, liliacea, linseed, millet, muskmelon, mustard, oat, oil palm, oilseed rape, papaya, peanut, pineapple, ornamental plants, Phaseolus, potato, rapeseed, rice, rye, ryegrass, safflower, sesame, sorghum, soybean, sugarbeet, sugarcane, sunflower, strawberry, tobacco, tomato, turfgrass, wheat and vegetable crops such as lettuce, celery, broccoli, cauliflower, cucurbits; fruit and nut trees, such as apple, pear, peach, orange, grapefruit, lemon, lime, almond, pecan, walnut, hazel; vines, such as grapes (e.g., a vineyard), kiwi, hops; fruit shrubs and brambles, such as raspberry, blackberry, gooseberry; forest trees, such as ash, pine, fir, maple, oak, chestnut, popular; with alfalfa, canola, castor bean, corn, cotton, crambe, flax, linseed, mustard, oil palm, oilseed rape, peanut, potato, rice, safflower, sesame, soybean, sugarbeet, sunflower, tobacco, tomato, and wheat. Plants that can be treated in accordance with the methods of the present invention include any crop plant, for example, forage crop, oilseed crop, grain crop, fruit crop, vegetable crop, fiber crop, spice crop, nut crop, turf crop, sugar crop, beverage crop, and forest crop. In certain instances, the crop plant that is treated in the method is a soybean plant. In other certain instances, the crop plant is wheat. In certain instances, the crop plant is corn. In certain instances, the crop plant is cotton. In certain instances, the crop plant is alfalfa. In certain instances, the crop plant is sugarbeet. In

certain instances, the crop plant is rice. In certain instances, the crop plant is potato. In certain instances, the crop plant is tomato.

In certain instances, the plant is a crop. Examples of such crop plants include, but are not limited to, monocotyledonous and dicotyledonous plants including, but not limited to, fodder or forage legumes, ornamental plants, food crops, trees, or shrubs selected from *Acer* spp., *Allium* spp., *Amaranthus* spp., *Ananas comosus*, *Apium graveolens*, *Arachis* spp., *Asparagus officinalis*, *Beta vulgaris*, *Brassica* spp. (e.g., *Brassica napus*, *Brassica rapa* ssp. (canola, oilseed rape, turnip rape), *Camellia sinensis*, *Canna indica*, *Cannabis saliva*, *Capsicum* spp., *Castanea* spp., *Cichorium endivia*, *Citrullus lanatus*, *Citrus* spp., *Cocos* spp., *Coffea* spp., *Coriandrum sativum*, *Corylus* spp., *Crataegus* spp., *Cucurbita* spp., *Cucumis* spp., *Daucus carota*, *Fagus* spp., *Ficus carica*, *Fragaria* spp., *Ginkgo biloba*, *Glycine* spp. (e.g., *Glycine max*, *Soja hispida* or *Soja max*), *Gossypium hirsutum*, *Helianthus* spp. (e.g., *Helianthus annuus*), *Hibiscus* spp., *Hordeum* spp. (e.g., *Hordeum vulgare*), *Ipomoea batatas*, *Juglans* spp., *Lactuca sativa*, *Linum usitatissimum*, *Litchi chinensis*, *Lotus* spp., *Luffa acutangula*, *Lupinus* spp., *Lycopersicon* spp. (e.g., *Lycopersicon esculentum*, *Lycopersicon lycopersicum*, *Lycopersicon pyriforme*), *Malus* spp., *Medicago sativa*, *Mentha* spp., *Miscanthus sinensis*, *Morus nigra*, *Musa* spp., *Nicotiana* spp., *Olea* spp., *Oryza* spp. (e.g., *Oryza sativa*, *Oryza latifolia*), *Panicum miliaceum*, *Panicum virgatum*, *Passiflora edulis*, *Petroselinum crispum*, *Phaseolus* spp., *Pinus* spp., *Pistacia vera*, *Pisum* spp., *Poa* spp., *Populus* spp., *Prunus* spp., *Pyrus communis*, *Quercus* spp., *Raphanus sativus*, *Rheum rhabarbarum*, *Ribes* spp., *Ricinus communis*, *Rubus* spp., *Saccharum* spp., *Salix* sp., *Sambucus* spp., *Secale cereale*, *Sesamum* spp., *Sinapis* spp., *Solanum* spp. (e.g., *Solanum tuberosum*, *Solanum integrifolium* or *Solanum lycopersicum*), *Sorghum bicolor*, *Sorghum halepense*, *Spinacia* spp., *Tamarindus indica*, *Theobroma cacao*, *Trifolium* spp., *Triticosecale rimpaui*, *Triticum* spp. (e.g., *Triticum aestivum*, *Triticum durum*, *Triticum turgidum*, *Triticum hybernum*, *Triticum macha*, *Triticum sativum* or *Triticum vulgare*), *Vaccinium* spp., *Vicia* spp., *Vigna* spp., *Viola odorata*, *Vitis* spp., and *Zea mays*. In certain embodiments, the crop plant is rice, oilseed rape, canola, soybean, corn (maize), cotton, sugarcane, alfalfa, sorghum, or wheat.

The plant or plant part for use in the present invention includes plants of any stage of plant development. In certain instances, the delivery can occur during the stages of germination, seedling growth, vegetative growth, and reproductive growth. In certain instances, delivery to

the plant occurs during vegetative and reproductive growth stages. In some instances, the composition is delivered to pollen of the plant. In some instances, the composition is delivered to a seed of the plant. In some instances, the composition is delivered to a protoplast of the plant. In some instances, the composition is delivered to a tissue of the plant. For example, the composition may be delivered to meristematic tissue of the plant (e.g., apical meristem, lateral meristem, or intercalary meristem). In some instances, the composition is delivered to permanent tissue of the plant (e.g., simple tissues (e.g., parenchyma, collenchyma, or sclerenchyma) or complex permanent tissue (e.g., xylem or phloem)). In some instances, the composition is delivered to a plant embryo. In some instances, the composition is delivered to a plant cell. The stages of vegetative and reproductive growth are also referred to herein as “adult” or “mature” plants.

In instances where the gene modifying system is delivered to a plant part, the plant part may be modified by the plant-modifying agent. Alternatively, the gene modifying system may be distributed to other parts of the plant (e.g., by the plant’s circulatory system) that are subsequently modified by the plant-modifying agent.

Administration and Delivery

The compositions and systems described herein may be used *in vitro* or *in vivo*. In some embodiments the system or components of the system are delivered to cells (e.g., mammalian cells, e.g., human cells), e.g., *in vitro* or *in vivo*. In some embodiments, the cells are eukaryotic cells, e.g., cells of a multicellular organism, e.g., a plant or an animal, e.g., a mammal (e.g., human, swine, bovine), a bird (e.g., poultry, such as chicken, turkey, or duck), or a fish. In some embodiments, the cells are non-human animal cells (e.g., a laboratory animal, a livestock animal, or a companion animal). In some embodiments, the cell is a stem cell (e.g., a hematopoietic stem cell), a fibroblast, or a T cell. In some embodiments, the cell is an immune cell, e.g., a T cell (e.g., a Treg, CD4, CD8, $\gamma\delta$, or memory T cell), B cell (e.g., memory B cell or plasma cell), or NK cell. In some embodiments, the cell is a non-dividing cell, e.g., a non-dividing fibroblast or non-dividing T cell. In some embodiments, the cell is an HSC and p53 is not upregulated or is upregulated by less than 10%, 5%, 2%, or 1%, e.g., as determined according to the method described in Example 30 of PCT/US2019/048607. The skilled artisan will understand that the

components of the gene modifying system may be delivered in the form of polypeptide, nucleic acid (e.g., DNA, RNA), and combinations thereof.

In one embodiment the system and/or components of the system are delivered as nucleic acid. For example, the gene modifying polypeptide may be delivered in the form of a DNA or
5 RNA encoding the polypeptide, and the template RNA may be delivered in the form of RNA or its complementary DNA to be transcribed into RNA. In some embodiments the system or components of the system are delivered on 1, 2, 3, 4, or more distinct nucleic acid molecules. In some embodiments the system or components of the system are delivered as a combination of DNA and RNA. In some embodiments the system or components of the system are delivered as
10 a combination of DNA and protein. In some embodiments the system or components of the system are delivered as a combination of RNA and protein. In some embodiments the gene modifying polypeptide is delivered as a protein.

In some embodiments the system or components of the system are delivered to cells, e.g. mammalian cells or human cells, using a vector. The vector may be, e.g., a plasmid or a virus.
15 In some embodiments, delivery is *in vivo*, *in vitro*, *ex vivo*, or *in situ*. In some embodiments the virus is an adeno associated virus (AAV), a lentivirus, or an adenovirus. In some embodiments the system or components of the system are delivered to cells with a viral-like particle or a virosome. In some embodiments the delivery uses more than one virus, viral-like particle or virosome.

20 In one embodiment, the compositions and systems described herein can be formulated in liposomes or other similar vesicles. Liposomes are spherical vesicle structures composed of a uni- or multilamellar lipid bilayer surrounding internal aqueous compartments and a relatively impermeable outer lipophilic phospholipid bilayer. Liposomes may be anionic, neutral or cationic. Liposomes are biocompatible, nontoxic, can deliver both hydrophilic and lipophilic
25 drug molecules, protect their cargo from degradation by plasma enzymes, and transport their load across biological membranes and the blood brain barrier (BBB) (see, e.g., Spuch and Navarro, Journal of Drug Delivery, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review).

Vesicles can be made from several different types of lipids; however, phospholipids are
30 most commonly used to generate liposomes as drug carriers. Methods for preparation of multilamellar vesicle lipids are known in the art (see for example U.S. Pat. No. 6,693,086, the

teachings of which relating to multilamellar vesicle lipid preparation are incorporated herein by reference). Although vesicle formation can be spontaneous when a lipid film is mixed with an aqueous solution, it can also be expedited by applying force in the form of shaking by using a homogenizer, sonicator, or an extrusion apparatus (see, e.g., Spuch and Navarro, *Journal of Drug*
5 *Delivery*, vol. 2011, Article ID 469679, 12 pages, 2011. doi:10.1155/2011/469679 for review). Extruded lipids can be prepared by extruding through filters of decreasing size, as described in Templeton et al., *Nature Biotech*, 15:647-652, 1997, the teachings of which relating to extruded lipid preparation are incorporated herein by reference.

A variety of nanoparticles can be used for delivery, such as a liposome, a lipid
10 nanoparticle, a cationic lipid nanoparticle, an ionizable lipid nanoparticle, a polymeric nanoparticle, a gold nanoparticle, a dendrimer, a cyclodextrin nanoparticle, a micelle, or a combination of the foregoing.

Lipid nanoparticles are an example of a carrier that provides a biocompatible and biodegradable delivery system for the pharmaceutical compositions described herein.
15 Nanostructured lipid carriers (NLCs) are modified solid lipid nanoparticles (SLNs) that retain the characteristics of the SLN, improve drug stability and loading capacity, and prevent drug leakage. Polymer nanoparticles (PNPs) are an important component of drug delivery. These nanoparticles can effectively direct drug delivery to specific targets and improve drug stability and controlled drug release. Lipid-polymer nanoparticles (PLNs), a type of carrier that
20 combines liposomes and polymers, may also be employed. These nanoparticles possess the complementary advantages of PNPs and liposomes. A PLN is composed of a core-shell structure; the polymer core provides a stable structure, and the phospholipid shell offers good biocompatibility. As such, the two components increase the drug encapsulation efficiency rate, facilitate surface modification, and prevent leakage of water-soluble drugs. For a review, see,
25 e.g., Li et al. 2017, *Nanomaterials* 7, 122; doi:10.3390/nano7060122.

Exosomes can also be used as drug delivery vehicles for the compositions and systems described herein. For a review, see Ha et al. July 2016. *Acta Pharmaceutica Sinica B*. Volume 6, Issue 4, Pages 287-296; <https://doi.org/10.1016/j.apsb.2016.02.001>.

Fusosomes interact and fuse with target cells, and thus can be used as delivery vehicles
30 for a variety of molecules. They generally consist of a bilayer of amphipathic lipids enclosing a lumen or cavity and a fusogen that interacts with the amphipathic lipid bilayer. The fusogen

component has been shown to be engineerable in order to confer target cell specificity for the fusion and payload delivery, allowing the creation of delivery vehicles with programmable cell specificity (see for example Patent Application WO2020014209, the teachings of which relating to fusosome design, preparation, and usage are incorporated herein by reference).

5 In some embodiments, the protein component(s) of the gene modifying system may be pre-associated with the template nucleic acid (e.g., template RNA). For example, in some embodiments, the gene modifying polypeptide may be first combined with the template nucleic acid (e.g., template RNA) to form a ribonucleoprotein (RNP) complex. In some embodiments, the RNP may be delivered to cells via, e.g., transfection, nucleofection, virus, vesicle, LNP,
10 exosome, fusosome.

A gene modifying system can be introduced into cells, tissues and multicellular organisms. In some embodiments the system or components of the system are delivered to the cells via mechanical means or physical means.

Formulation of protein therapeutics is described in Meyer (Ed.), *Therapeutic Protein*
15 *Drug Products: Practical Approaches to formulation in the Laboratory, Manufacturing, and the Clinic*, Woodhead Publishing Series (2012).

Tissue Specific Activity/Administration

In some embodiments, a system described herein can make use of one or more feature (e.g., a promoter or microRNA binding site) to limit activity in off-target cells or tissues.

20 In some embodiments, a nucleic acid described herein (e.g., a template RNA or a DNA encoding a template RNA) comprises a promoter sequence, e.g., a tissue specific promoter sequence. In some embodiments, the tissue-specific promoter is used to increase the target-cell specificity of a gene modifying system. For instance, the promoter can be chosen on the basis that it is active in a target cell type but not active in (or active at a lower level in) a non-target
25 cell type. Thus, even if the promoter integrated into the genome of a non-target cell, it would not drive expression (or only drive low level expression) of an integrated gene. A system having a tissue-specific promoter sequence in the template RNA may also be used in combination with a microRNA binding site, e.g., in the template RNA or a nucleic acid encoding a gene modifying protein, e.g., as described herein. A system having a tissue-specific promoter sequence in the
30 template RNA may also be used in combination with a DNA encoding a gene modifying

polypeptide, driven by a tissue-specific promoter, e.g., to achieve higher levels of gene modifying protein in target cells than in non-target cells. In some embodiments, e.g., for liver indications, a tissue-specific promoter is selected from Table 3 of WO2020014209, incorporated herein by reference.

5 In some embodiments, a nucleic acid described herein (e.g., a template RNA or a DNA encoding a template RNA) comprises a microRNA binding site. In some embodiments, the microRNA binding site is used to increase the target-cell specificity of a gene modifying system. For instance, the microRNA binding site can be chosen on the basis that is recognized by a miRNA that is present in a non-target cell type, but that is not present (or is present at a reduced
10 level relative to the non-target cell) in a target cell type. Thus, when the template RNA is present in a non-target cell, it would be bound by the miRNA, and when the template RNA is present in a target cell, it would not be bound by the miRNA (or bound but at reduced levels relative to the non-target cell). While not wishing to be bound by theory, binding of the miRNA to the template RNA may interfere with its activity, e.g., may interfere with insertion of the
15 heterologous object sequence into the genome. Accordingly, the system would edit the genome of target cells more efficiently than it edits the genome of non-target cells, e.g., the heterologous object sequence would be inserted into the genome of target cells more efficiently than into the genome of non-target cells, or an insertion or deletion is produced more efficiently in target cells than in non-target cells. A system having a microRNA binding site in the template RNA (or
20 DNA encoding it) may also be used in combination with a nucleic acid encoding a gene modifying polypeptide, wherein expression of the gene modifying polypeptide is regulated by a second microRNA binding site, e.g., as described herein. In some embodiments, e.g., for liver indications, a miRNA is selected from Table 4 of WO2020014209, incorporated herein by reference.

25 In some embodiments, the template RNA comprises a microRNA sequence, an siRNA sequence, a guide RNA sequence, or a piwi RNA sequence.

Promoters

In some embodiments, one or more promoter or enhancer elements are operably linked to a nucleic acid encoding a gene modifying protein or a template nucleic acid, e.g., that controls
30 expression of the heterologous object sequence. In certain embodiments, the one or more

promoter or enhancer elements comprise cell-type or tissue specific elements. In some embodiments, the promoter or enhancer is the same or derived from the promoter or enhancer that naturally controls expression of the heterologous object sequence. For example, the ornithine transcarbamylase promoter and enhancer may be used to control expression of the ornithine transcarbamylase gene in a system or method provided by the invention for correcting ornithine transcarbamylase deficiencies. In some embodiments, the promoter is a promoter of Table 16 or 17 or a functional fragment or variant thereof.

Exemplary tissue specific promoters that are commercially available can be found, for example, at a uniform resource locator (e.g., www.invivogen.com/tissue-specific-promoters). In some embodiments, a promoter is a native promoter or a minimal promoter, e.g., which consists of a single fragment from the 5' region of a given gene. In some embodiments, a native promoter comprises a core promoter and its natural 5' UTR. In some embodiments, the 5' UTR comprises an intron. In other embodiments, these include composite promoters, which combine promoter elements of different origins or were generated by assembling a distal enhancer with a minimal promoter of the same origin.

Exemplary cell or tissue specific promoters are provided in the tables, below, and exemplary nucleic acid sequences encoding them are known in the art and can be readily accessed using a variety of resources, such as the NCBI database, including RefSeq, as well as the Eukaryotic Promoter Database ([//epd.epfl.ch//index.php](http://epd.epfl.ch//index.php)).

Table 16. Exemplary cell or tissue-specific promoters

Promoter	Target cells
B29 Promoter	B cells
CD14 Promoter	Monocytic Cells
CD43 Promoter	Leukocytes and platelets
CD45 Promoter	Hematopoietic cells
CD68 promoter	macrophages
Desmin promoter	muscle cells
Elastase-1 promoter	pancreatic acinar cells
Endoglin promoter	endothelial cells
fibronectin promoter	differentiating cells, healing tissue
Flt-1 promoter	endothelial cells
GFAP promoter	Astrocytes
GPIIB promoter	megakaryocytes

Promoter	Target cells
ICAM-2 Promoter	Endothelial cells
INF-Beta promoter	Hematopoietic cells
Mb promoter	muscle cells
Nphs1 promoter	podocytes
OG-2 promoter	Osteoblasts, Odontoblasts
SP-B promoter	Lung
Syn1 promoter	Neurons
WASP promoter	Hematopoietic cells
SV40/bAlb promoter	Liver
SV40/bAlb promoter	Liver
SV40/Cd3 promoter	Leukocytes and platelets
SV40/CD45 promoter	hematopoietic cells
NSE/RU5' promoter	Mature Neurons

Table 17. Additional exemplary cell or tissue-specific promoters

Promoter	Gene Description	Gene Specificity
APOA2	Apolipoprotein A-II	Hepatocytes (from hepatocyte progenitors)
SERPINA1 (hAAT)	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (also named alpha 1 anti-tryps in)	Hepatocytes (from definitive endoderm stage)
CYP3A	Cytochrome P450, family 3, subfamily A, polypeptide	Mature Hepatocytes
MIR122	MicroRNA 122	Hepatocytes (from early stage embryonic liver cells) and endoderm
Pancreatic specific promoters		
Promoter	Gene Description	Gene Specificity
INS	Insulin	Pancreatic beta cells (from definitive endoderm stage)
IRS2	Insulin receptor substrate 2	Pancreatic beta cells
Pdx1	Pancreatic and duodenal homeobox 1	Pancreas (from definitive endoderm stage)
Alx3	Aristaless-like homeobox 3	Pancreatic beta cells (from definitive endoderm stage)
Ppy	Pancreatic polypeptide	PP pancreatic cells (gamma cells)
Cardiac specific promoters		
Promoter	Gene Description	Gene Specificity
Myh6 (aMHC)	Myosin, heavy chain 6, cardiac muscle, alpha	Late differentiation marker of cardiac muscle cells (atrial specificity)
MYL2 (MLC-2v)	Myosin, light chain 2, regulatory, cardiac, slow	Late differentiation marker of cardiac muscle cells (ventricular specificity)
ITNNI3 (cTnI)	Troponin I type 3 (cardiac)	Cardiomyocytes (from immature state)
ITNNI3 (cTnI)	Troponin I type 3 (cardiac)	Cardiomyocytes (from immature state)

Promoter	Gene Description	Gene Specificity
NPPA (ANF)	Natriuretic peptide precursor A (also named Atrial Natriuretic Factor)	Atrial specificity in adult cells
Slc8a1 (Ncx1)	Solute carrier family 8 (sodium/calcium exchanger), member 1	Cardiomyocytes from early developmental stages
CNS specific promoters		
SYN1 (hSyn)	Synapsin I	Neurons
GFAP	Glial fibrillary acidic protein	Astrocytes
INA	Internexin neuronal intermediate filament protein, alpha (a-internexin)	Neuroprogenitors
NES	Nestin	Neuroprogenitors and ectoderm
MOBP	Myelin-associated oligodendrocyte basic protein	Oligodendrocytes
MBP	Myelin basic protein	Oligodendrocytes
TH	Tyrosine hydroxylase	Dopaminergic neurons
FOXA2 (HNF3 beta)	Forkhead box A2	Dopaminergic neurons (also used as a marker of endoderm)
Skin specific promoters		
Promoter	Gene Description	Gene Specificity
FLG	Filaggrin	Keratinocytes from granular layer
K14	Keratin 14	Keratinocytes from granular and basal layers
TGM3	Transglutaminase 3	Keratinocytes from granular layer
Immune cell specific promoters		
Promoter	Gene Description	Gene Specificity
ITGAM (CD11B)	Integrin, alpha M (complement component 3 receptor 3 subunit)	Monocytes, macrophages , granulocytes , natural killer cells
Urogenital cell specific promoters		
Promoter	Gene Description	Gene Specificity
Pbsn	Probasin	Prostatic epithelium
Upk2	Uroplakin 2	Bladder
Sbp	Spermine binding protein	Prostate
Fer114	Fer-1-like 4	Bladder
Endothelial cell specific promoters		
Promoter	Gene Description	Gene Specificity
ENG	Endoglin	Endothelial cells
Pluripotent and embryonic cell specific promoters		
Promoter	Gene Description	Gene Specificity
Oct4 (POU5F1)	POU class 5 homeobox 1	Pluripotent cells (germ cells, ES cells, iPS cells)
NANOG	Nanog homeobox	Pluripotent cells (ES cells, iPS cells)
Synthetic Oct4	Synthetic promoter based on a Oct-4 core enhancer element	Pluripotent cells (ES cells, iPS cells)
T brachyury	Brachyury	Mesoderm
NES	Nestin	Neuroprogenitors and Ectoderm
SOX17	SRY (sex determining region Y)-box 17	Endoderm

Promoter	Gene Description	Gene Specificity
FOXA2 (HNFJ beta)	Forkhead box A2	Endoderm (also used as a marker of dopaminergic neurons)
MIR122	MicroRNA 122	Endoderm and hepatocytes (from early stage embryonic liver cells~

Depending on the host/vector system utilized, any of a number of suitable transcription and translation control elements, including constitutive and inducible **promoters**, transcription enhancer elements, transcription terminators, etc. may be used in the expression vector (see e.g.,
5 Bitter et al. (1987) *Methods in Enzymology*, 153:516-544; incorporated herein by reference in its entirety).

In some embodiments, a nucleic acid encoding a **gene modifying** protein or template nucleic acid is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element may, in some embodiment, be functional in
10 either a eukaryotic cell, e.g., a mammalian cell; or a prokaryotic cell (e.g., bacterial or archaeal cell). In some embodiments, a nucleotide sequence encoding a polypeptide is operably linked to multiple control elements, e.g., that allow expression of the nucleotide sequence encoding the polypeptide in both prokaryotic and eukaryotic cells.

For illustration purposes, examples of spatially restricted **promoters** include, but are not
15 limited to, neuron-specific **promoters**, adipocyte-specific **promoters**, cardiomyocyte-specific **promoters**, smooth muscle-specific **promoters**, photoreceptor-specific **promoters**, etc. Neuron-specific spatially restricted **promoters** include, but are not limited to, a neuron-specific enolase (NSE) promoter (see, e.g., EMBL HSENO2, X51956), an aromatic amino acid decarboxylase (AADC) promoter, a neurofilament promoter (see, e.g., GenBank HUMNFL, L04147); a
20 synapsin promoter (see, e.g., GenBank HUMSYNIB, M55301); a thy-1 promoter (see, e.g., Chen et al. (1987) *Cell* 51:7-19; and Llewellyn, et al. (2010) *Nat. Med.* 16(10):1161-1166), a serotonin receptor promoter (see, e.g., GenBank S62283); a tyrosine hydroxylase promoter (TH) (see, e.g., Oh et al. (2009) *Gene Ther* 16:437; Sasaoka et al. (1992) *Mol. Brain Res.* 16:274; Boundy et al. (1998) *J. Neurosci.* 18:9989; and Kaneda et al. (1991) *Neuron* 6:583-594); a GnRH promoter
25 (see, e.g., Radovick et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:3402-3406), an L7 promoter (see, e.g., Oberdick et al. (1990) *Science* 248:223-226); a DNMT promoter (see, e.g., Bartge et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:3648-3652); an enkephalin promoter (see, e.g., Comb et al. (1988) *EMBO J.* 17:3793-3805); a myelin basic protein (MBP) promoter; a Ca²⁺-calmodulin-dependent protein kinase II-alpha (CamKII α) promoter (see, e.g., Mayford et al.

(1996) *Proc. Natl. Acad. Sci. USA* 93:13250; and Casanova et al. (2001) *Genesis* 31:37); a CMV enhancer/platelet-derived growth factor- β promoter (see, e.g., Liu et al. (2004) *Gene Therapy* 11:52-60); and the like.

Adipocyte-specific spatially restricted promoters include, but are not limited to, the aP2 gene promoter/enhancer, e.g., a region from -5.4 kb to +21 bp of a human aP2 gene (see, e.g., Tozzo et al. (1997) *Endocrinol.* 138:1604; Ross et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:9590; and Pavjani et al. (2005) *Nat. Med.* 11:797); a glucose transporter-4 (GLUT4) promoter (see, e.g., Knight et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:14725); a fatty acid translocase (FAT/CD36) promoter (see, e.g., Kuriki et al. (2002) *Biol. Pharm. Bull.* 25:1476; and Sato et al. (2002) *J. Biol. Chem.* 277:15703); a stearoyl-CoA desaturase-1 (SCD1) promoter (Tabor et al. (1999) *J. Biol. Chem.* 274:20603); a leptin promoter (see, e.g., Mason et al. (1998) *Endocrinol.* 139:1013; and Chen et al. (1999) *Biochem. Biophys. Res. Comm.* 262:187); an adiponectin promoter (see, e.g., Kita et al. (2005) *Biochem. Biophys. Res. Comm.* 331:484; and Chakrabarti (2010) *Endocrinol.* 151:2408); an adipisin promoter (see, e.g., Platt et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:7490); a resistin promoter (see, e.g., Seo et al. (2003) *Molec. Endocrinol.* 17:1522); and the like.

Cardiomyocyte-specific spatially restricted promoters include, but are not limited to, control sequences derived from the following genes: myosin light chain-2, α -myosin heavy chain, AE3, cardiac troponin C, cardiac actin, and the like. Franz et al. (1997) *Cardiovasc. Res.* 35:560-566; Robbins et al. (1995) *Ann. N.Y. Acad. Sci.* 752:492-505; Linn et al. (1995) *Circ. Res.* 76:584-591; Parmacek et al. (1994) *Mol. Cell. Biol.* 14:1870-1885; Hunter et al. (1993) *Hypertension* 22:608-617; and Sartorelli et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4047-4051.

Smooth muscle-specific spatially restricted promoters include, but are not limited to, an SM22 α promoter (see, e.g., Akyürek et al. (2000) *Mol. Med.* 6:983; and U.S. Pat. No. 7,169,874); a smoothelin promoter (see, e.g., WO 2001/018048); an α -smooth muscle actin promoter; and the like. For example, a 0.4 kb region of the SM22 α promoter, within which lie two CArG elements, has been shown to mediate vascular smooth muscle cell-specific expression (see, e.g., Kim, et al. (1997) *Mol. Cell. Biol.* 17, 2266-2278; Li, et al., (1996) *J. Cell Biol.* 132, 849-859; and Moessler, et al. (1996) *Development* 122, 2415-2425).

Photoreceptor-specific spatially restricted promoters include, but are not limited to, a rhodopsin promoter; a rhodopsin kinase promoter (Young et al. (2003) *Ophthalmol. Vis. Sci.*

44:4076); a beta phosphodiesterase gene promoter (Nicoud et al. (2007) J. Gene Med. 9:1015); a retinitis pigmentosa gene promoter (Nicoud et al. (2007) supra); an interphotoreceptor retinoid-binding protein (IRBP) gene enhancer (Nicoud et al. (2007) supra); an IRBP gene promoter (Yokoyama et al. (1992) Exp Eye Res. 55:225); and the like.

5 In some embodiments, a gene modifying system, e.g., DNA encoding a gene modifying polypeptide, DNA encoding a template RNA, or DNA or RNA encoding a heterologous object sequence, is designed such that one or more elements is operably linked to a tissue-specific promoter, e.g., a promoter that is active in T-cells. In further embodiments, the T-cell active promoter is inactive in other cell types, e.g., B-cells, NK cells. In some embodiments, the T-cell
10 active promoter is derived from a promoter for a gene encoding a component of the T-cell receptor, e.g., TRAC, TRBC, TRGC, TRDC. In some embodiments, the T-cell active promoter is derived from a promoter for a gene encoding a component of a T-cell-specific cluster of differentiation protein, e.g., CD3, e.g., CD3D, CD3E, CD3G, CD3Z. In some embodiments, T-cell-specific promoters in gene modifying systems are discovered by comparing publicly
15 available gene expression data across cell types and selecting promoters from the genes with enhanced expression in T-cells. In some embodiments, promoters may be selecting depending on the desired expression breadth, e.g., promoters that are active in T-cells only, promoters that are active in NK cells only, promoters that are active in both T-cells and NK cells.

Cell-specific promoters known in the art may be used to direct expression of a gene
20 modifying protein, e.g., as described herein. Nonlimiting exemplary mammalian cell-specific promoters have been characterized and used in mice expressing Cre recombinase in a cell-specific manner. Certain nonlimiting exemplary mammalian cell-specific promoters are listed in Table 1 of US9845481, incorporated herein by reference.

In some embodiments, a vector as described herein comprises an expression cassette.
25 Typically, an expression cassette comprises the nucleic acid molecule of the instant invention operatively linked to a promoter sequence. For example, a promoter is operatively linked with a coding sequence when it is capable of affecting the expression of that coding sequence (e.g., the coding sequence is under the transcriptional control of the promoter). Encoding sequences can be operatively linked to regulatory sequences in sense or antisense orientation. In certain
30 embodiments, the promoter is a heterologous promoter. In certain embodiments, an expression cassette may comprise additional elements, for example, an intron, an enhancer, a

polyadenylation site, a woodchuck response element (WRE), and/or other elements known to affect expression levels of the encoding sequence. A **promoter** typically controls the expression of a coding sequence or functional RNA. In certain embodiments, a **promoter** sequence comprises proximal and more distal upstream elements and can further comprise an **enhancer** element. An **enhancer** can typically stimulate **promoter** activity and may be an innate element of the **promoter** or a heterologous element inserted to enhance the level or tissue-specificity of a **promoter**. In certain embodiments, the **promoter** is derived in its entirety from a native **gene**. In certain embodiments, the **promoter** is composed of different elements derived from different naturally occurring promoters. In certain embodiments, the **promoter** comprises a synthetic nucleotide sequence. It will be understood by those skilled in the art that different promoters will direct the expression of a **gene** in different tissues or cell types, or at different stages of development, or in response to different environmental conditions or to the presence or the absence of a drug or transcriptional co-factor. Ubiquitous, cell-type-specific, tissue-specific, developmental stage-specific, and conditional promoters, for example, drug-responsive promoters (e.g., tetracycline-responsive promoters) are well known to those of skill in the art. Exemplary promoters include, but are not limited to, the phosphoglycerate kinase (PKG) promoter, CAG (composite of the CMV **enhancer** the chicken beta actin promoter (CBA) and the rabbit beta globin intron), NSE (neuronal specific enolase), synapsin or NeuN promoters, the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), a herpes simplex virus (HSV) promoter, a cytomegalovirus (CMV) promoter such as the CMV immediate early promoter region (CMVIE), SFFV promoter, rous sarcoma virus (RSV) promoter, synthetic promoters, hybrid promoters, and the like. Other promoters can be of human origin or from other species, including from mice. Common promoters include, e.g., the human cytomegalovirus (CMV) immediate early **gene promoter**, the SV40 early promoter, the Rous sarcoma virus long terminal repeat, [beta]- actin, rat insulin promoter, the phosphoglycerate kinase promoter, the human alpha-1 antitrypsin (hAAT) promoter, the transthyretin promoter, the TBG promoter and other liver-specific promoters, the desmin promoter and similar muscle-specific promoters, the EF1 -alpha promoter, hybrid promoters with multi-tissue specificity, promoters specific for neurons like synapsin and glyceraldehyde-3 -phosphate dehydrogenase promoter, all of which are promoters well known and readily available to those of skill in the art, can be used to obtain high-level expression of the

coding sequence of interest. In addition, sequences derived from non-viral genes, such as the murine metallothionein gene, will also find use herein. Such promoter sequences are commercially available from, e.g., Stratagene (San Diego, CA). Additional exemplary promoter sequences are described, for example, in WO2018213786A1 (incorporated by reference herein in its entirety).

In some embodiments, the apolipoprotein E enhancer (ApoE) or a functional fragment thereof is used, e.g., to drive expression in the liver. In some embodiments, two copies of the ApoE enhancer or a functional fragment thereof are used. In some embodiments, the ApoE enhancer or functional fragment thereof is used in combination with a promoter, e.g., the human alpha-1 antitrypsin (hAAT) promoter.

In some embodiments, the regulatory sequences impart tissue-specific gene expression capabilities. In some cases, the tissue-specific regulatory sequences bind tissue-specific transcription factors that induce transcription in a tissue specific manner. Various tissue-specific regulatory sequences (e.g., promoters, enhancers, etc.) are known in the art. Exemplary tissue-specific regulatory sequences include, but are not limited to, the following tissue-specific promoters: a liver-specific thyroxine binding globulin (TBG) promoter, an insulin promoter, a glucagon promoter, a somatostatin promoter, a pancreatic polypeptide (PPY) promoter, a synapsin-1 (Syn) promoter, a creatine kinase (MCK) promoter, a mammalian desmin (DES) promoter, a α -myosin heavy chain (α -MHC) promoter, or a cardiac Troponin T (cTnT) promoter. Other exemplary promoters include Beta-actin promoter, hepatitis B virus core promoter, Sandig et al., Gene Ther., 3:1002-9 (1996); alpha-fetoprotein (AFP) promoter, Arbuthnot et al., Hum. Gene Ther., 7:1503-14 (1996), bone osteocalcin promoter (Stein et al., Mol. Biol. Rep., 24:185-96 (1997)), bone sialoprotein promoter (Chen et al., J. Bone Miner. Res., 11:654-64 (1996)), CD2 promoter (Hansal et al., J. Immunol., 161:1063-8 (1998); immunoglobulin heavy chain promoter; T cell receptor α -chain promoter, neuronal such as neuron-specific enolase (NSE) promoter (Andersen et al., Cell. Mol. Neurobiol., 13:503-15 (1993)), neurofilament light-chain gene promoter (Piccioli et al., Proc. Natl. Acad. Sci. USA, 88:5611-5 (1991)), and the neuron-specific vgf gene promoter (Piccioli et al., Neuron, 15:373-84 (1995)), and others. Additional exemplary promoter sequences are described, for example, in U.S. Patent No. 10300146 (incorporated herein by reference in its entirety). In some embodiments, a tissue-specific regulatory element, e.g., a tissue-specific promoter, is selected from one known to be operably

linked to a gene that is highly expressed in a given tissue, e.g., as measured by RNA-seq or protein expression data, or a combination thereof. Methods for analyzing tissue specificity by expression are taught in Fagerberg et al. *Mol Cell Proteomics* 13(2):397-406 (2014), which is incorporated herein by reference in its entirety.

5 In some embodiments, a vector described herein is a multicistronic expression construct. Multicistronic expression constructs include, for example, constructs harboring a first expression cassette, e.g. comprising a first promoter and a first encoding nucleic acid sequence, and a second expression cassette, e.g. comprising a second promoter and a second encoding nucleic acid sequence. Such multicistronic expression constructs may, in some instances, be particularly
10 useful in the delivery of non-translated gene products, such as hairpin RNAs, together with a polypeptide, for example, a **gene modifying** polypeptide and **gene modifying** template. In some embodiments, multicistronic expression constructs may exhibit reduced expression levels of one or more of the included transgenes, for example, because of promoter interference or the presence of incompatible nucleic acid elements in close proximity. If a multicistronic expression
15 construct is part of a viral vector, the presence of a self-complementary nucleic acid sequence may, in some instances, interfere with the formation of structures necessary for viral reproduction or packaging.

In some embodiments, the sequence encodes an RNA with a hairpin. In some embodiments, the hairpin RNA is a guide RNA, a template RNA, a shRNA, or a microRNA. In
20 some embodiments, the first promoter is an RNA polymerase I promoter. In some embodiments, the first promoter is an RNA polymerase II promoter. In some embodiments, the second promoter is an RNA polymerase III promoter. In some embodiments, the second promoter is a U6 or H1 promoter.

Without wishing to be bound by theory, multicistronic expression constructs may not
25 achieve optimal expression levels as compared to expression systems containing only one cistron. One of the suggested causes of lower expression levels achieved with multicistronic expression constructs comprising two or more promoter elements is the phenomenon of promoter interference (see, e.g., Curtin J A, Dane A P, Swanson A, Alexander I E, Ginn S L. *Bidirectional promoter interference between two widely used internal heterologous promoters in a late-generation lentiviral construct*. *Gene Ther.* 2008 March; 15(5):384-90; and Martin-Duque P, Jezzard S, Kaftansis L, Vassaux G. *Direct comparison of the insulating properties of two genetic*
30

elements in an adenoviral vector containing two different expression cassettes. Hum Gene Ther. 2004 October; 15(10):995-1002; both references incorporated herein by reference for disclosure of promoter interference phenomenon). In some embodiments, the problem of promoter interference may be overcome, e.g., by producing multicistronic expression constructs comprising only one promoter driving transcription of multiple encoding nucleic acid sequences separated by internal ribosomal entry sites, or by separating cistrons comprising their own promoter with transcriptional insulator elements. In some embodiments, single-promoter driven expression of multiple cistrons may result in uneven expression levels of the cistrons. In some embodiments, a promoter cannot efficiently be isolated and isolation elements may not be compatible with some gene transfer vectors, for example, some retroviral vectors.

MicroRNAs

MicroRNAs (miRNAs) and other small interfering nucleic acids generally regulate gene expression via target RNA transcript cleavage/degradation or translational repression of the target messenger RNA (mRNA). miRNAs may, in some instances, be natively expressed, typically as final 19-25 non-translated RNA products. miRNAs generally exhibit their activity through sequence-specific interactions with the 3' untranslated regions (UTR) of target mRNAs. These endogenously expressed miRNAs may form hairpin precursors that are subsequently processed into an miRNA duplex, and further into a mature single stranded miRNA molecule. This mature miRNA generally guides a multiprotein complex, miRISC, which identifies target 3' UTR regions of target mRNAs based upon their complementarity to the mature miRNA. Useful transgene products may include, for example, miRNAs or miRNA binding sites that regulate the expression of a linked polypeptide. A non-limiting list of miRNA genes; the products of these genes and their homologues are useful as transgenes or as targets for small interfering nucleic acids (e.g., miRNA sponges, antisense oligonucleotides), e.g., in methods such as those listed in US10300146, 22:25-25:48, are herein incorporated by reference. In some embodiments, one or more binding sites for one or more of the foregoing miRNAs are incorporated in a transgene, e.g., a transgene delivered by an rAAV vector, e.g., to inhibit the expression of the transgene in one or more tissues of an animal harboring the transgene. In some embodiments, a binding site may be selected to control the expression of a transgene in a tissue specific manner. For example, binding sites for the liver-specific miR-122 may be incorporated into a transgene to

inhibit expression of that transgene in the liver. Additional exemplary miRNA sequences are described, for example, in U.S. Patent No. 10,300,146 (incorporated herein by reference in its entirety).

5 An miR inhibitor or miRNA inhibitor is generally an agent that blocks miRNA expression and/or processing. Examples of such agents include, but are not limited to, microRNA antagonists, microRNA specific antisense, microRNA sponges, and microRNA oligonucleotides (double-stranded, hairpin, short oligonucleotides) that inhibit miRNA interaction with a Drosha complex. MicroRNA inhibitors, e.g., miRNA sponges, can be expressed in cells from transgenes (e.g., as described in Ebert, M. S. Nature Methods, Epub Aug. 10 12, 2007; incorporated by reference herein in its entirety). In some embodiments, microRNA sponges, or other miR inhibitors, are used with the AAVs. microRNA sponges generally specifically inhibit miRNAs through a complementary heptameric seed sequence. In some embodiments, an entire family of miRNAs can be silenced using a single sponge sequence. Other methods for silencing miRNA function (derepression of miRNA targets) in cells will be apparent 15 to one of ordinary skill in the art.

In some embodiments, a gene modifying system, template RNA, or polypeptide described herein is administered to or is active in (e.g., is more active in) a target tissue, e.g., a first tissue. In some embodiments, the gene modifying system, template RNA, or polypeptide is not administered to or is less active in (e.g., not active in) a non-target tissue. In some 20 embodiments, a gene modifying system, template RNA, or polypeptide described herein is useful for modifying DNA in a target tissue, e.g., a first tissue, (e.g., and not modifying DNA in a non-target tissue).

In some embodiments, a gene modifying system comprises (a) a polypeptide described herein or a nucleic acid encoding the same, (b) a template nucleic acid (e.g., template RNA) 25 described herein, and (c) one or more first tissue-specific expression-control sequences specific to the target tissue, wherein the one or more first tissue-specific expression-control sequences specific to the target tissue are in operative association with (a), (b), or (a) and (b), wherein, when associated with (a), (a) comprises a nucleic acid encoding the polypeptide.

In some embodiments, the nucleic acid in (b) comprises RNA.

30 In some embodiments, the nucleic acid in (b) comprises DNA.

In some embodiments, the nucleic acid in (b): (i) is single-stranded or comprises a single-stranded segment, e.g., is single-stranded DNA or comprises a single-stranded segment and one or more double stranded segments; (ii) has inverted terminal repeats; or (iii) both (i) and (ii).

In some embodiments, the nucleic acid in (b) is double-stranded or comprises a double-stranded segment.

In some embodiments, (a) comprises a nucleic acid encoding the polypeptide.

In some embodiments, the nucleic acid in (a) comprises RNA.

In some embodiments, the nucleic acid in (a) comprises DNA.

In some embodiments, the nucleic acid in (a): (i) is single-stranded or comprises a single-stranded segment, e.g., is single-stranded DNA or comprises a single-stranded segment and one or more double stranded segments; (ii) has inverted terminal repeats; or (iii) both (i) and (ii).

In some embodiments, the nucleic acid in (a) is double-stranded or comprises a double-stranded segment.

In some embodiments, the nucleic acid in (a), (b), or (a) and (b) is linear.

In some embodiments, the nucleic acid in (a), (b), or (a) and (b) is circular, e.g., a plasmid or minicircle.

In some embodiments, the heterologous object sequence is in operative association with a first promoter.

In some embodiments, the one or more first tissue-specific expression-control sequences comprises a tissue specific promoter.

In some embodiments, the tissue-specific promoter comprises a first promoter in operative association with: (i) the heterologous object sequence, (ii) a nucleic acid encoding the retroviral RT, or (iii) (i) and (ii).

In some embodiments, the one or more first tissue-specific expression-control sequences comprises a tissue-specific microRNA recognition sequence in operative association with: (i) the heterologous object sequence, (ii) a nucleic acid encoding the retroviral RT domain, or (iii) (i) and (ii).

In some embodiments, a system comprises a tissue-specific promoter, and the system further comprises one or more tissue-specific microRNA recognition sequences, wherein: (i) the tissue specific promoter is in operative association with: (I) the heterologous object sequence, (II) a nucleic acid encoding the retroviral RT domain, or (III) (I) and (II); and/or (ii) the one or

more tissue-specific microRNA recognition sequences are in operative association with: (I) the heterologous object sequence, (II) a nucleic acid encoding the retroviral RT, or (III) (I) and (II).

In some embodiments, wherein (a) comprises a nucleic acid encoding the polypeptide, the nucleic acid comprises a promoter in operative association with the nucleic acid encoding the polypeptide.

In some embodiments, the nucleic acid encoding the polypeptide comprises one or more second tissue-specific expression-control sequences specific to the target tissue in operative association with the polypeptide coding sequence.

In some embodiments, the one or more second tissue-specific expression-control sequences comprises a tissue specific promoter.

In some embodiments, the tissue-specific promoter is the promoter in operative association with the nucleic acid encoding the polypeptide.

In some embodiments, the one or more second tissue-specific expression-control sequences comprises a tissue-specific microRNA recognition sequence.

In some embodiments, the promoter in operative association with the nucleic acid encoding the polypeptide is a tissue-specific promoter, the system further comprising one or more tissue-specific microRNA recognition sequences.

In some embodiments, a nucleic acid component of a system provided by the invention is a sequence (e.g., encoding the polypeptide or comprising a heterologous object sequence) flanked by untranslated regions (UTRs) that modify protein expression levels. Various 5' and 3' UTRs can affect protein expression. For example, in some embodiments, the coding sequence may be preceded by a 5' UTR that modifies RNA stability or protein translation. In some embodiments, the sequence may be followed by a 3' UTR that modifies RNA stability or translation. In some embodiments, the sequence may be preceded by a 5' UTR and followed by a 3' UTR that modify RNA stability or translation. In some embodiments, the 5' and/or 3' UTR may be selected from the 5' and 3' UTRs of complement factor 3 (C3)

(CACTCCTCCCCATCCTCTCCCTCTGTCCCTCTGTCCCTCTGACCCTGCACTGTCCCAG CACC) or orosomucoid 1 (ORM1)

(CAGGACACAGCCTTGGATCAGGACAGAGACTTGGGGGCCATCCTGCCCCCTCCAACC CGACATGTGTACCTCAGCTTTTCCCTCACTTGCATCAATAAAGCTTCTGTGTTTGA ACAGCTAA) (Asrani et al. RNA Biology 2018). In certain embodiments, the 5' UTR is the 5'

UTR from C3 and the 3' UTR is the 3' UTR from ORM1. In certain embodiments, a 5' UTR and 3' UTR for protein expression, e.g., mRNA (or DNA encoding the RNA) for a gene modifying polypeptide or heterologous object sequence, comprise optimized expression sequences. In some embodiments, the 5' UTR comprises

- 5 GGGAAAU AAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGAGCCACC and/or the 3' UTR comprising
 UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCC
 AGCCCCUCCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGA,
 e.g., as described in Richner et al. *Cell* 168(6): P1114-1125 (2017), the sequences of which are
 10 incorporated herein by reference.

In some embodiments, a 5' and/or 3' UTR may be selected to enhance protein expression. In some embodiments, a 5' and/or 3' UTR may be selected to modify protein expression such that overproduction inhibition is minimized. In some embodiments, UTRs are around a coding sequence, e.g., outside the coding sequence and in other embodiments proximal
 15 to the coding sequence. In some embodiments, additional regulatory elements (e.g., miRNA binding sites, cis-regulatory sites) are included in the UTRs.

In some embodiments, an open reading frame of a gene modifying system, e.g., an ORF of an mRNA (or DNA encoding an mRNA) encoding a gene modifying polypeptide or one or more ORFs of an mRNA (or DNA encoding an mRNA) of a heterologous object sequence, is
 20 flanked by a 5' and/or 3' untranslated region (UTR) that enhances the expression thereof. In some embodiments, the 5' UTR of an mRNA component (or transcript produced from a DNA component) of the system comprises the sequence 5'-
 GGGAAAU AAGAGAGAAAAGAAGAGUAAGAAGAAAUUAAGAGCCACC-3'. In some
 embodiments, the 3' UTR of an mRNA component (or transcript produced from a DNA
 25 component) of the system comprises the sequence 5'-
 UGAUAAUAGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCC
 AGCCCCUCCUCCCCUCCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGA-
 3'. This combination of 5' UTR and 3' UTR has been shown to result in desirable expression of
 an operably linked ORF by Richner et al. *Cell* 168(6): P1114-1125 (2017), the teachings and
 30 sequences of which are incorporated herein by reference. In some embodiments, a system described herein comprises a DNA encoding a transcript, wherein the DNA comprises the

corresponding 5' UTR and 3' UTR sequences, with T substituting for U in the above-listed sequence). In some embodiments, a DNA vector used to produce an RNA component of the system further comprises a promoter upstream of the 5' UTR for initiating *in vitro* transcription, e.g., a T7, T3, or SP6 promoter. The 5' UTR above begins with GGG, which is a suitable start
5 for optimizing transcription using T7 RNA polymerase. For tuning transcription levels and altering the transcription start site nucleotides to fit alternative 5' UTRs, the teachings of Davidson et al. *Pac Symp Biocomput* 433-443 (2010) describe T7 promoter variants, and the methods of discovery thereof, that fulfill both of these traits.

Viral vectors and components thereof

10 Viruses are a useful source of delivery vehicles for the systems described herein, in addition to a source of relevant enzymes or domains as described herein, e.g., as sources of polymerases and polymerase functions used herein, e.g., DNA-dependent DNA polymerase, RNA-dependent RNA polymerase, RNA-dependent DNA polymerase, DNA-dependent RNA polymerase, reverse transcriptase. Some enzymes, e.g., reverse transcriptases, may have
15 multiple activities, e.g., be capable of both RNA-dependent DNA polymerization and DNA-dependent DNA polymerization, e.g., first and second strand synthesis. In some embodiments, the virus used as a gene modifying delivery system or a source of components thereof may be selected from a group as described by Baltimore *Bacteriol Rev* 35(3):235-241 (1971).

In some embodiments, the virus is selected from a Group I virus, e.g., is a DNA virus and
20 packages dsDNA into virions. In some embodiments, the Group I virus is selected from, e.g., Adenoviruses, Herpesviruses, Poxviruses.

In some embodiments, the virus is selected from a Group II virus, e.g., is a DNA virus and packages ssDNA into virions. In some embodiments, the Group II virus is selected from, e.g., Parvoviruses. In some embodiments, the parvovirus is a dependoparvovirus, e.g., an adeno-
25 associated virus (AAV).

In some embodiments, the virus is selected from a Group III virus, e.g., is an RNA virus and packages dsRNA into virions. In some embodiments, the Group III virus is selected from, e.g., Reoviruses. In some embodiments, one or both strands of the dsRNA contained in such virions is a coding molecule able to serve directly as mRNA upon transduction into a host cell,

e.g., can be directly translated into protein upon transduction into a host cell without requiring any intervening nucleic acid replication or polymerization steps.

In some embodiments, the virus is selected from a Group IV virus, e.g., is an RNA virus and packages ssRNA(+) into virions. In some embodiments, the Group IV virus is selected
5 from, e.g., Coronaviruses, Picornaviruses, Togaviruses. In some embodiments, the ssRNA(+) contained in such virions is a coding molecule able to serve directly as mRNA upon transduction into a host cell, e.g., can be directly translated into protein upon transduction into a host cell without requiring any intervening nucleic acid replication or polymerization steps.

In some embodiments, the virus is selected from a Group V virus, e.g., is an RNA virus
10 and packages ssRNA(-) into virions. In some embodiments, the Group V virus is selected from, e.g., Orthomyxoviruses, Rhabdoviruses. In some embodiments, an RNA virus with an ssRNA(-) genome also carries an enzyme inside the virion that is transduced to host cells with the viral genome, e.g., an RNA-dependent RNA polymerase, capable of copying the ssRNA(-) into ssRNA(+) that can be translated directly by the host.

In some embodiments, the virus is selected from a Group VI virus, e.g., is a retrovirus
15 and packages ssRNA(+) into virions. In some embodiments, the Group VI virus is selected from, e.g., retroviruses. In some embodiments, the retrovirus is a lentivirus, e.g., HIV-1, HIV-2, SIV, BIV. In some embodiments, the retrovirus is a spumavirus, e.g., a foamy virus, e.g., HFV, SFV, BFV. In some embodiments, the ssRNA(+) contained in such virions is a coding molecule
20 able to serve directly as mRNA upon transduction into a host cell, e.g., can be directly translated into protein upon transduction into a host cell without requiring any intervening nucleic acid replication or polymerization steps. In some embodiments, the ssRNA(+) is first reverse transcribed and copied to generate a dsDNA genome intermediate from which mRNA can be transcribed in the host cell. In some embodiments, an RNA virus with an ssRNA(+) genome
25 also carries an enzyme inside the virion that is transduced to host cells with the viral genome, e.g., an RNA-dependent DNA polymerase, capable of copying the ssRNA(+) into dsDNA that can be transcribed into mRNA and translated by the host. In some embodiments, the reverse transcriptase from a Group VI retrovirus is incorporated as the reverse transcriptase domain of a gene modifying polypeptide.

30 In some embodiments, the virus is selected from a Group VII virus, e.g., is a retrovirus and packages dsRNA into virions. In some embodiments, the Group VII virus is selected from,

e.g., Hepadnaviruses. In some embodiments, one or both strands of the dsRNA contained in such virions is a coding molecule able to serve directly as mRNA upon transduction into a host cell, e.g., can be directly translated into protein upon transduction into a host cell without requiring any intervening nucleic acid replication or polymerization steps. In some
5 embodiments, one or both strands of the dsRNA contained in such virions is first reverse transcribed and copied to generate a dsDNA genome intermediate from which mRNA can be transcribed in the host cell. In some embodiments, an RNA virus with a dsRNA genome also carries an enzyme inside the virion that is transduced to host cells with the viral genome, e.g., an RNA-dependent DNA polymerase, capable of copying the dsRNA into dsDNA that can be
10 transcribed into mRNA and translated by the host. In some embodiments, the reverse transcriptase from a Group VII retrovirus is incorporated as the reverse transcriptase domain of a gene modifying polypeptide.

In some embodiments, virions used to deliver nucleic acid in this invention may also carry enzymes involved in the process of gene modification. For example, a retroviral virion
15 may contain a reverse transcriptase domain that is delivered into a host cell along with the nucleic acid. In some embodiments, an RNA template may be associated with a gene modifying polypeptide within a virion, such that both are co-delivered to a target cell upon transduction of the nucleic acid from the viral particle. In some embodiments, the nucleic acid in a virion may comprise DNA, e.g., linear ssDNA, linear dsDNA, circular ssDNA, circular dsDNA, minicircle
20 DNA, dbDNA, ceDNA. In some embodiments, the nucleic acid in a virion may comprise RNA, e.g., linear ssRNA, linear dsRNA, circular ssRNA, circular dsRNA. In some embodiments, a viral genome may circularize upon transduction into a host cell, e.g., a linear ssRNA molecule may undergo a covalent linkage to form a circular ssRNA, a linear dsRNA molecule may undergo a covalent linkage to form a circular dsRNA or one or more circular ssRNA. In some
25 embodiments, a viral genome may replicate by rolling circle replication in a host cell. In some embodiments, a viral genome may comprise a single nucleic acid molecule, e.g., comprise a non-segmented genome. In some embodiments, a viral genome may comprise two or more nucleic acid molecules, e.g., comprise a segmented genome. In some embodiments, a nucleic acid in a virion may be associated with one or proteins. In some embodiments, one or more proteins in a
30 virion may be delivered to a host cell upon transduction. In some embodiments, a natural virus may be adapted for nucleic acid delivery by the addition of virion packaging signals to the target

nucleic acid, wherein a host cell is used to package the target nucleic acid containing the packaging signals.

In some embodiments, a virion used as a delivery vehicle may comprise a commensal human virus. In some embodiments, a virion used as a delivery vehicle may comprise an anellovirus, the use of which is described in WO2018232017A1, which is incorporated herein by reference in its entirety.

AAV Administration

In some embodiments, an adeno-associated virus (AAV) is used in conjunction with the system, template nucleic acid, and/or polypeptide described herein. In some embodiments, an AAV is used to deliver, administer, or package the system, template nucleic acid, and/or polypeptide described herein. In some embodiments, the AAV is a recombinant AAV (rAAV).

In some embodiments, a system comprises (a) a polypeptide described herein or a nucleic acid encoding the same, (b) a template nucleic acid (e.g., template RNA) described herein, and (c) one or more first tissue-specific expression-control sequences specific to the target tissue, wherein the one or more first tissue-specific expression-control sequences specific to the target tissue are in operative association with (a), (b), or (a) and (b), wherein, when associated with (a), (a) comprises a nucleic acid encoding the polypeptide.

In some embodiments, a system described herein further comprises a first recombinant adeno-associated virus (rAAV) capsid protein; wherein the at least one of (a) or (b) is associated with the first rAAV capsid protein, wherein at least one of (a) or (b) is flanked by AAV inverted terminal repeats (ITRs).

In some embodiments, (a) and (b) are associated with the first rAAV capsid protein.

In some embodiments, (a) and (b) are on a single nucleic acid.

In some embodiments, the system further comprises a second rAAV capsid protein, wherein at least one of (a) or (b) is associated with the second rAAV capsid protein, and wherein the at least one of (a) or (b) associated with the second rAAV capsid protein is different from the at least one of (a) or (b) is associated with the first rAAV capsid protein.

In some embodiments, the at least one of (a) or (b) is associated with the first or second rAAV capsid protein is dispersed in the interior of the first or second rAAV capsid protein, which first or second rAAV capsid protein is in the form of an AAV capsid particle.

In some embodiments, the system further comprises a nanoparticle, wherein the nanoparticle is associated with at least one of (a) or (b).

In some embodiments, (a) and (b), respectively are associated with: a) a first rAAV capsid protein and a second rAAV capsid protein; b) a nanoparticle and a first rAAV capsid protein; c) a first rAAV capsid protein; d) a first adenovirus capsid protein; e) a first nanoparticle and a second nanoparticle; or f) a first nanoparticle.

Viral vectors are useful for delivering all or part of a system provided by the invention, e.g., for use in methods provided by the invention. Systems derived from different viruses have been employed for the delivery of polypeptides or nucleic acids; for example: integrase-deficient lentivirus, adenovirus, adeno-associated virus (AAV), herpes simplex virus, and baculovirus (reviewed in Hodge et al. *Hum Gene Ther* 2017; Narayanavari et al. *Crit Rev Biochem Mol Biol* 2017; Boehme et al. *Curr Gene Ther* 2015).

Adenoviruses are common viruses that have been used as gene delivery vehicles given well-defined biology, genetic stability, high transduction efficiency, and ease of large-scale production (see, for example, review by Lee et al. *Genes & Diseases* 2017). They possess linear dsDNA genomes and come in a variety of serotypes that differ in tissue and cell tropisms. In order to prevent replication of infectious virus in recipient cells, adenovirus genomes used for packaging are deleted of some or all endogenous viral proteins, which are provided in trans in viral production cells. This renders the genomes helper-dependent, meaning they can only be replicated and packaged into viral particles in the presence of the missing components provided by so-called helper functions. A helper-dependent adenovirus system with all viral ORFs removed may be compatible with packaging foreign DNA of up to ~37 kb (Parks et al. *J Virol* 1997). In some embodiments, an adenoviral vector is used to deliver DNA corresponding to the polypeptide or template component of the gene modifying system, or both are contained on separate or the same adenoviral vector. In some embodiments, the adenovirus is a helper-dependent adenovirus (HD-AdV) that is incapable of self-packaging. In some embodiments, the adenovirus is a high-capacity adenovirus (HC-AdV) that has had all or a substantial portion of endogenous viral ORFs deleted, while retaining the necessary sequence components for packaging into adenoviral particles. For this type of vector, the only adenoviral sequences required for genome packaging are noncoding sequences: the inverted terminal repeats (ITRs) at both ends and the packaging signal at the 5'-end (Jager et al. *Nat Protoc* 2009). In some

embodiments, the adenoviral genome also comprises stuffer DNA to meet a minimal genome size for optimal production and stability (see, for example, Hausl et al. Mol Ther 2010). In some embodiments, an adenovirus is used to deliver a gene modifying system to the liver.

In some embodiments, an adenovirus is used to deliver a gene modifying system to HSCs, e.g., HDAd5/35++. HDAd5/35++ is an adenovirus with modified serotype 35 fibers that de-target the vector from the liver (Wang et al. Blood Adv 2019). In some embodiments, the adenovirus that delivers a gene modifying system to HSCs utilizes a receptor that is expressed specifically on primitive HSCs, e.g., CD46.

Adeno-associated viruses (AAV) belong to the parvoviridae family and more specifically constitute the dependoparvovirus genus. The AAV genome is composed of a linear single-stranded DNA molecule which contains approximately 4.7 kilobases (kb) and consists of two major open reading frames (ORFs) encoding the non-structural Rep (replication) and structural Cap (capsid) proteins. A second ORF within the cap gene was identified that encodes the assembly-activating protein (AAP). The DNAs flanking the AAV coding regions are two cis-acting inverted terminal repeat (ITR) sequences, approximately 145 nucleotides in length, with interrupted palindromic sequences that can be folded into energetically stable hairpin structures that function as primers of DNA replication. In addition to their role in DNA replication, the ITR sequences have been shown to be involved in viral DNA integration into the cellular genome, rescue from the host genome or plasmid, and encapsidation of viral nucleic acid into mature virions (Muzyczka, (1992) Curr. Top. Micro. Immunol. 158:97-129). In some embodiments, one or more gene modifying nucleic acid components is flanked by ITRs derived from AAV for viral packaging. See, e.g., WO2019113310.

In some embodiments, one or more components of the gene modifying system are carried via at least one AAV vector. In some embodiments, the at least one AAV vector is selected for tropism to a particular cell, tissue, organism. In some embodiments, the AAV vector is pseudotyped, e.g., AAV2/8, wherein AAV2 describes the design of the construct but the capsid protein is replaced by that from AAV8. It is understood that any of the described vectors could be pseudotype derivatives, wherein the capsid protein used to package the AAV genome is derived from that of a different AAV serotype. Without wishing to be limited in vector choice, a list of exemplary AAV serotypes can be found in Table 18. In some embodiments, an AAV to be

employed for gene modifying may be evolved for novel cell or tissue tropism as has been demonstrated in the literature (e.g., Davidsson et al. Proc Natl Acad Sci U S A 2019).

In some embodiments, the AAV delivery vector is a vector which has two AAV inverted terminal repeats (ITRs) and a nucleotide sequence of interest (for example, a sequence coding for a gene modifying polypeptide or a DNA template, or both), each of said ITRs having an interrupted (or noncontiguous) palindromic sequence, i.e., a sequence composed of three segments: a first segment and a last segment that are identical when read 5'→3' but hybridize when placed against each other, and a segment that is different that separates the identical segments. See, for example, WO2012123430.

Conventionally, AAV virions with capsids are produced by introducing a plasmid or plasmids encoding the rAAV or scAAV genome, Rep proteins, and Cap proteins (Grimm et al, 1998). Upon introduction of these helper plasmids in trans, the AAV genome is “rescued” (i.e., released and subsequently recovered) from the host genome, and is further encapsidated to produce infectious AAV. In some embodiments, one or more gene modifying nucleic acids are packaged into AAV particles by introducing the ITR-flanked nucleic acids into a packaging cell in conjunction with the helper functions.

In some embodiments, the AAV genome is a so called self-complementary genome (referred to as scAAV), such that the sequence located between the ITRs contains both the desired nucleic acid sequence (e.g., DNA encoding the gene modifying polypeptide or template, or both) in addition to the reverse complement of the desired nucleic acid sequence, such that these two components can fold over and self-hybridize. In some embodiments, the self-complementary modules are separated by an intervening sequence that permits the DNA to fold back on itself, e.g., forms a stem-loop. An scAAV has the advantage of being poised for transcription upon entering the nucleus, rather than being first dependent on ITR priming and second-strand synthesis to form dsDNA. In some embodiments, one or more gene modifying components is designed as an scAAV, wherein the sequence between the AAV ITRs contains two reverse complementing modules that can self-hybridize to create dsDNA.

In some embodiments, nucleic acid (e.g., encoding a polypeptide, or a template, or both) delivered to cells is closed-ended, linear duplex DNA (CELiD DNA or ceDNA). In some embodiments, ceDNA is derived from the replicative form of the AAV genome (Li et al. PLoS One 2013). In some embodiments, the nucleic acid (e.g., encoding a polypeptide, or a template

DNA, or both) is flanked by ITRs, e.g., AAV ITRs, wherein at least one of the ITRs comprises a terminal resolution site and a replication protein binding site (sometimes referred to as a replicative protein binding site). In some embodiments, the ITRs are derived from an adeno-associated virus, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, or a combination thereof. In some embodiments, the ITRs are symmetric. In some embodiments, the ITRs are asymmetric. In some embodiments, at least one Rep protein is provided to enable replication of the construct. In some embodiments, the at least one Rep protein is derived from an adeno-associated virus, e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, or a combination thereof. In some embodiments, ceDNA is generated by providing a production cell with (i) DNA flanked by ITRs, e.g., AAV ITRs, and (ii) components required for ITR-dependent replication, e.g., AAV proteins Rep78 and Rep52 (or nucleic acid encoding the proteins). In some embodiments, ceDNA is free of any capsid protein, e.g., is not packaged into an infectious AAV particle. In some embodiments, ceDNA is formulated into LNPs (see, for example, WO2019051289A1).

In some embodiments, the ceDNA vector consists of two self-complementary sequences, e.g., asymmetrical or symmetrical or substantially symmetrical ITRs as defined herein, flanking said expression cassette, wherein the ceDNA vector is not associated with a capsid protein. In some embodiments, the ceDNA vector comprises two self-complementary sequences found in an AAV genome, where at least one ITR comprises an operative Rep-binding element (RBE) (also sometimes referred to herein as “RBS”) and a terminal resolution site (trs) of AAV or a functional variant of the RBE. See, for example, WO2019113310.

In some embodiments, the AAV genome comprises two genes that encode four replication proteins and three capsid proteins, respectively. In some embodiments, the genes are flanked on either side by 145-bp inverted terminal repeats (ITRs). In some embodiments, the virion comprises up to three capsid proteins (Vp1, Vp2, and/or Vp3), e.g., produced in a 1:1:10 ratio. In some embodiments, the capsid proteins are produced from the same open reading frame and/or from differential splicing (Vp1) and alternative translational start sites (Vp2 and Vp3, respectively). Generally, Vp3 is the most abundant subunit in the virion and participates in receptor recognition at the cell surface defining the tropism of the virus. In some embodiments, Vp1 comprises a phospholipase domain, e.g., which functions in viral infectivity, in the N-terminus of Vp1.

In some embodiments, packaging capacity of the viral vectors limits the size of the gene modifying system that can be packaged into the vector. For example, the packaging capacity of the AAVs can be about 4.5 kb (e.g., about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, or 6.0 kb), e.g., including one or two inverted terminal repeats (ITRs), e.g., 145 base ITRs.

5 In some embodiments, recombinant AAV (rAAV) comprises cis-acting 145-bp ITRs flanking vector transgene cassettes, e.g., providing up to 4.5 kb for packaging of foreign DNA. Subsequent to infection, rAAV can, in some instances, express a fusion protein of the invention and persist without integration into the host genome by existing episomally in circular head-to-tail concatemers. rAAV can be used, for example, *in vitro* and *in vivo*. In some embodiments,
10 AAV-mediated gene delivery requires that the length of the coding sequence of the gene is equal or greater in size than the wild-type AAV genome.

AAV delivery of genes that exceed this size and/or the use of large physiological regulatory elements can be accomplished, for example, by dividing the protein(s) to be delivered into two or more fragments. In some embodiments, the N-terminal fragment is fused to an intein-
15 N sequence. In some embodiments, the C-terminal fragment is fused to an intein-C sequence. In embodiments, the fragments are packaged into two or more AAV vectors.

In some embodiments, dual AAV vectors are generated by splitting a large transgene expression cassette in two separate halves (5' and 3' ends, or head and tail), e.g., wherein each half of the cassette is packaged in a single AAV vector (of <5 kb). The re-assembly of the full-
20 length transgene expression cassette can, in some embodiments, then be achieved upon co-infection of the same cell by both dual AAV vectors. In some embodiments, co-infection is followed by one or more of: (1) homologous recombination (HR) between 5' and 3' genomes (dual AAV overlapping vectors); (2) ITR-mediated tail-to-head concatemerization of 5' and 3' genomes (dual AAV trans-splicing vectors); and/or (3) a combination of these two mechanisms
25 (dual AAV hybrid vectors). In some embodiments, the use of dual AAV vectors *in vivo* results in the expression of full-length proteins. In some embodiments, the use of the dual AAV vector platform represents an efficient and viable gene transfer strategy for transgenes of greater than about 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0 kb in size. In some embodiments, AAV vectors can also be used to transduce cells with target nucleic acids, e.g., in the *in vitro*
30 production of nucleic acids and peptides. In some embodiments, AAV vectors can be used for *in vivo* and *ex vivo* gene therapy procedures (see, e.g., West et al., Virology 160:38-47 (1987); U.S.

Patent No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest.94:1351 (1994); each of which is incorporated herein by reference in their entirety). The construction of recombinant AAV vectors is described in a number of publications, including U.S. Patent No.5,173,414; Tratschin et al., Mol. Cell. Biol.5:3251- 3260
5 (1985); Tratschin, et al., Mol. Cell. Biol.4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol.63:03822-3828 (1989) (incorporated by reference herein in their entirety).

In some embodiments, a gene modifying polypeptide described herein (e.g., with or without one or more guide nucleic acids) can be delivered using AAV, lentivirus, adenovirus or
10 other plasmid or viral vector types, in particular, using formulations and doses from, for example, U.S. Patent No. 8,454,972 (formulations, doses for adenovirus), U.S. Patent No.8,404,658 (formulations, doses for AAV) and U.S. Patent No.5,846,946 (formulations, doses for DNA plasmids) and from clinical trials and publications regarding the clinical trials involving lentivirus, AAV and adenovirus. For example, for AAV, the route of administration, formulation
15 and dose can be as described in U.S. Patent No.8,454,972 and as in clinical trials involving AAV. For adenovirus, the route of administration, formulation and dose can be as described in U.S. Patent No.8,404,658 and as in clinical trials involving adenovirus. For plasmid delivery, the route of administration, formulation and dose can be as described in U.S. Patent No.5,846,946 and as in clinical studies involving plasmids. Doses can be based on or extrapolated to an
20 average 70 kg individual (e.g. a male adult human), and can be adjusted for patients, subjects, mammals of different weight and species. Frequency of administration is within the ambit of the medical or veterinary practitioner (e.g., physician, veterinarian), depending on usual factors including the age, sex, general health, other conditions of the patient or subject and the particular condition or symptoms being addressed. In some embodiments, the viral vectors can be injected
25 into the tissue of interest. For cell-type specific gene modifying, the expression of the gene modifying polypeptide and optional guide nucleic acid can, in some embodiments, be driven by a cell-type specific promoter.

In some embodiments, AAV allows for low toxicity, for example, due to the purification method not requiring ultracentrifugation of cell particles that can activate the immune response.

30 In some embodiments, AAV allows low probability of causing insertional mutagenesis, for example, because it does not substantially integrate into the host genome.

In some embodiments, AAV has a packaging limit of about 4.4, 4.5, 4.6, 4.7, or 4.75 kb. In some embodiments, a gene modifying polypeptide-encoding sequence, promoter, and transcription terminator can fit into a single viral vector. SpCas9 (4.1 kb) may, in some instances, be difficult to package into AAV. Therefore, in some embodiments, a gene modifying

5 polypeptide coding sequence is used that is shorter in length than other gene modifying polypeptide coding sequences or base editors. In some embodiments, the gene modifying polypeptide encoding sequences are less than about 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2 kb, or 1.5 kb.

10 An AAV can be AAV1, AAV2, AAV5 or any combination thereof. In some embodiments, the type of AAV is selected with respect to the cells to be targeted; e.g., AAV serotypes 1, 2, 5 or a hybrid capsid AAV1, AAV2, AAV5 or any combination thereof can be selected for targeting brain or neuronal cells; or AAV4 can be selected for targeting cardiac tissue. In some embodiments, AAV8 is selected for delivery to the liver. Exemplary AAV

15 serotypes as to these cells are described, for example, in Grimm, D. et al, J. Virol. 82: 5887-5911 (2008) (incorporated herein by reference in its entirety). In some embodiments, AAV refers all serotypes, subtypes, and naturally-occurring AAV as well as recombinant AAV. AAV may be used to refer to the virus itself or a derivative thereof. In some embodiments, AAV includes AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV6.2, AAV7, AAVrh.64Rl,

20 AAVhu.37, AAVrh.8, AAVrh.32.33, AAV8, AAV9, AAV-DJ, AAV2/8, AAVrhIO, AAVLK03, AV10, AAV11, AAV 12, rhIO, and hybrids thereof, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. The genomic sequences of various serotypes of AAV, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits are known in the art. Such sequences may be found in the literature

25 or in public databases such as GenBank. Additional exemplary AAV serotypes are listed in Table 18.

Table 18. Exemplary AAV serotypes.

Target Tissue	Vehicle	Reference
Liver	AAV (AAV8 ¹ , AAVrh.8 ¹ , AAVhu.37 ¹ , AAV2/8, AAV2/rh10 ² , AAV9, AAV2, NP40 ³ , NP59 ^{2,3} , AAV3B ⁵ , AAV-DJ ⁴ , AAV-LK01 ⁴ , AAV-LK02 ⁴ , AAV-LK03 ⁴ , AAV-LK19 ⁴ , AAV5 ⁷ , Adenovirus (Ad5, HC-AdV ⁶)	1. Wang <i>et al.</i> , <i>Mol. Ther.</i> 18 , 118–25 (2010) 2. Ginn <i>et al.</i> , <i>JHEP Reports</i> , 100065 (2019) 3. Paulk <i>et al.</i> , <i>Mol. Ther.</i> 26 , 289–303 (2018). 4. L. Lisowski <i>et al.</i> , <i>Nature</i> . 506 , 382–6 (2014). 5. L. Wang <i>et al.</i> , <i>Mol. Ther.</i> 23 , 1877–87 (2015). 6. Hausl <i>Mol Ther</i> (2010)

		7. Davidoff <i>et al.</i> , <i>Mol. Ther.</i> 11, 875-88 (2005)
Lung	AAV (AAV4, AAV5, AAV6 ¹ , AAV9, H22 ²) Adenovirus (Ad5, Ad3, Ad21, Ad14) ³	1. Duncan <i>et al.</i> , <i>Mol Ther Methods Clin Dev</i> (2018) 2. Cooney <i>et al.</i> , <i>Am J Respir Cell Mol Biol</i> (2019) 3. Li <i>et al.</i> , <i>Mol Ther Methods Clin Dev</i> (2019)
Skin	AAV (AAV6 ¹ , AAV-LK19 ²)	1. Petek <i>et al.</i> , <i>Mol. Ther.</i> (2010) 2. L. Lisowski <i>et al.</i> , <i>Nature</i> . 506 , 382-6 (2014).
HSCs	Adenovirus (HDA5/35 ⁴)	Wang <i>et al.</i> , <i>Blood Adv</i> (2019)

In some embodiments, a pharmaceutical composition (e.g., comprising an AAV as described herein) has less than 10% empty capsids, less than 8% empty capsids, less than 7% empty capsids, less than 5% empty capsids, less than 3% empty capsids, or less than 1 % empty capsids. In some embodiments, the pharmaceutical composition has less than about 5% empty capsids. In some embodiments, the number of empty capsids is below the limit of detection. In some embodiments, it is advantageous for the pharmaceutical composition to have low amounts of empty capsids, e.g., because empty capsids may generate an adverse response (e.g., immune response, inflammatory response, liver response, and/or cardiac response), e.g., with little or no substantial therapeutic benefit.

In some embodiments, the residual host cell protein (rHCP) in the pharmaceutical composition is less than or equal to 100 ng/ml rHCP per 1×10^{13} vg/ml, e.g., less than or equal to 40 ng/ml rHCP per 1×10^{13} vg/ml or 1-50 ng/ml rHCP per 1×10^{13} vg/ml. In some embodiments, the pharmaceutical composition comprises less than 10 ng rHCP per 1.0×10^{13} vg, or less than 5 ng rHCP per 1.0×10^{13} vg, less than 4 ng rHCP per 1.0×10^{13} vg, or less than 3 ng rHCP per 1.0×10^{13} vg, or any concentration in between. In some embodiments, the residual host cell DNA (hcDNA) in the pharmaceutical composition is less than or equal to 5×10^6 pg/ml hcDNA per 1×10^{13} vg/ml, less than or equal to 1.2×10^6 pg/ml hcDNA per 1×10^{13} vg/ml, or 1×10^5 pg/ml hcDNA per 1×10^{13} vg/ml. In some embodiments, the residual host cell DNA in said pharmaceutical composition is less than 5.0×10^5 pg per 1×10^{13} vg, less than 2.0×10^5 pg per 1.0×10^{13} vg, less than 1.1×10^5 pg per 1.0×10^{13} vg, less than 1.0×10^5 pg hcDNA per 1.0×10^{13} vg, less than 0.9×10^5 pg hcDNA per 1.0×10^{13} vg, less than 0.8×10^5 pg hcDNA per 1.0×10^{13} vg, or any concentration in between.

In some embodiments, the residual plasmid DNA in the pharmaceutical composition is less than or equal to 1.7×10^5 pg/ml per 1.0×10^{13} vg/ml, or 1×10^5 pg/ml per $1 \times 1.0 \times 10^{13}$ vg/ml, or 1.7×10^6 pg/ml per 1.0×10^{13} vg/ml. In some embodiments, the residual DNA plasmid in the pharmaceutical composition is less than 10.0×10^5 pg by 1.0×10^{13} vg, less than $8.0 \times$

10⁵ pg by 1.0 x 10¹³ vg or less than 6.8 x 10⁵ pg by 1.0 x 10¹³ vg. In embodiments, the pharmaceutical composition comprises less than 0.5 ng per 1.0 x 10¹³ vg, less than 0.3 ng per 1.0 x 10¹³ vg, less than 0.22 ng per 1.0 x 10¹³ vg or less than 0.2 ng per 1.0 x 10¹³ vg or any intermediate concentration of bovine serum albumin (BSA). In embodiments, the benzonase in the pharmaceutical composition is less than 0.2 ng by 1.0 x 10¹³ vg, less than 0.1 ng by 1.0 x 10¹³ vg, less than 0.09 ng by 1.0 x 10¹³ vg, less than 0.08 ng by 1.0 x 10¹³ vg or any intermediate concentration. In embodiments, Poloxamer 188 in the pharmaceutical composition is about 10 to 150 ppm, about 15 to 100 ppm or about 20 to 80 ppm. In embodiments, the cesium in the pharmaceutical composition is less than 50 pg / g (ppm), less than 30 pg / g (ppm) or less than 20 pg / g (ppm) or any intermediate concentration.

In embodiments, the pharmaceutical composition comprises total impurities, e.g., as determined by SDS-PAGE, of less than 10%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, or any percentage in between. In embodiments, the total purity, e.g., as determined by SDS-PAGE, is greater than 90%, greater than 92%, greater than 93%, greater than 94%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or any percentage in between. In embodiments, no single unnamed related impurity, e.g., as measured by SDS-PAGE, is greater than 5%, greater than 4%, greater than 3% or greater than 2%, or any percentage in between. In embodiments, the pharmaceutical composition comprises a percentage of filled capsids relative to total capsids (e.g., peak 1 + peak 2 as measured by analytical ultracentrifugation) of greater than 85%, greater than 86%, greater than 87%, greater than 88%, greater than 89%, greater than 90%, greater than 91%, greater than 91.9%, greater than 92%, greater than 93%, or any percentage in between. In embodiments of the pharmaceutical composition, the percentage of filled capsids measured in peak 1 by analytical ultracentrifugation is 20-80%, 25-75%, 30-75%, 35-75%, or 37.4-70.3%. In embodiments of the pharmaceutical composition, the percentage of filled capsids measured in peak 2 by analytical ultracentrifugation is 20-80%, 20-70%, 22-65%, 24-62%, or 24.9-60.1%.

In one embodiment, the pharmaceutical composition comprises a genomic titer of 1.0 to 5.0 x 10¹³ vg / mL, 1.2 to 3.0 x 10¹³ vg / mL or 1.7 to 2.3 x 10¹³ vg / mL. In one embodiment, the pharmaceutical composition exhibits a biological load of less than 5 CFU / mL, less than 4 CFU / mL, less than 3 CFU / mL, less than 2 CFU / mL or less than 1 CFU / mL or any intermediate contraction. In embodiments, the amount of endotoxin according to USP, for example, USP

<85> (incorporated by reference in its entirety) is less than 1.0 EU / mL, less than 0.8 EU / mL or less than 0.75 EU / mL. In embodiments, the osmolarity of a pharmaceutical composition according to USP, for example, USP <785> (incorporated by reference in its entirety) is 350 to 450 mOsm / kg, 370 to 440 mOsm / kg or 390 to 430 mOsm / kg. In embodiments, the pharmaceutical composition contains less than 1200 particles that are greater than 25 μ m per container, less than 1000 particles that are greater than 25 μ m per container, less than 500 particles that are greater than 25 μ m per container or any intermediate value. In embodiments, the pharmaceutical composition contains less than 10,000 particles that are greater than 10 μ m per container, less than 8000 particles that are greater than 10 μ m per container or less than 600 particles that are greater than 10 μ m per container.

In one embodiment, the pharmaceutical composition has a genomic titer of 0.5 to 5.0 x 10¹³ vg / mL, 1.0 to 4.0 x 10¹³ vg / mL, 1.5 to 3.0 x 10¹³ vg / ml or 1.7 to 2.3 x 10¹³ vg / ml. In one embodiment, the pharmaceutical composition described herein comprises one or more of the following: less than about 0.09 ng benzonase per 1.0 x 10¹³ vg, less than about 30 pg / g (ppm) of cesium, about 20 to 80 ppm Poloxamer 188, less than about 0.22 ng BSA per 1.0 x 10¹³ vg, less than about 6.8 x 10⁻⁵ pg of residual DNA plasmid per 1.0 x 10¹³ vg, less than about 1.1 x 10⁻⁵ pg of residual hcDNA per 1.0 x 10¹³ vg, less than about 4 ng of rHCP per 1.0 x 10¹³ vg, pH 7.7 to 8.3, about 390 to 430 mOsm / kg, less than about 600 particles that are > 25 μ m in size per container, less than about 6000 particles that are > 10 μ m in size per container, about 1.7 x 10¹³ - 2.3 x 10¹³ vg / mL genomic titer, infectious titer of about 3.9 x 10⁸ to 8.4 x 10¹⁰ IU per 1.0 x 10¹³ vg, total protein of about 100-300 pg per 1.0 x 10¹³ vg, mean survival of >24 days in A7SMA mice with about 7.5 x 10¹³ vg / kg dose of viral vector, about 70 to 130% relative potency based on an *in vitro* cell based assay and / or less than about 5% empty capsid. In various embodiments, the pharmaceutical compositions described herein comprise any of the viral particles discussed here, retain a potency of between $\pm$ 20%, between $\pm$ 15%, between $\pm$ 10% or within $\pm$ 5% of a reference standard. In some embodiments, potency is measured using a suitable *in vitro* cell assay or *in vivo* animal model.

Additional methods of preparation, characterization, and dosing AAV particles are taught in WO2019094253, which is incorporated herein by reference in its entirety.

Additional rAAV constructs that can be employed consonant with the invention include those described in Wang et al 2019, available at: [//doi.org/10.1038/s41573-019-0012-9](https://doi.org/10.1038/s41573-019-0012-9), including Table 1 thereof, which is incorporated by reference in its entirety.

Lipid Nanoparticles

5 The methods and systems provided herein may employ any suitable carrier or delivery modality, including, in certain embodiments, lipid nanoparticles (LNPs). Lipid nanoparticles, in some embodiments, comprise one or more ionic lipids, such as non-cationic lipids (e.g., neutral or anionic, or zwitterionic lipids); one or more conjugated lipids (such as PEG-conjugated lipids or lipids conjugated to polymers described in Table 5 of WO2019217941; incorporated herein by
10 reference in its entirety); one or more sterols (e.g., cholesterol); and, optionally, one or more targeting molecules (e.g., conjugated receptors, receptor ligands, antibodies); or combinations of the foregoing.

 Lipids that can be used in nanoparticle formations (e.g., lipid nanoparticles) include, for example those described in Table 4 of WO2019217941, which is incorporated by reference—
15 e.g., a lipid-containing nanoparticle can comprise one or more of the lipids in Table 4 of WO2019217941. Lipid nanoparticles can include additional elements, such as polymers, such as the polymers described in Table 5 of WO2019217941, incorporated by reference.

 In some embodiments, conjugated lipids, when present, can include one or more of PEG-diacylglycerol (DAG) (such as 1-(monomethoxy-polyethyleneglycol)-2,3- dimyristoylglycerol
20 (PEG-DMG)), PEG-dialkylxypropyl (DAA), PEG-phospholipid, PEG- ceramide (Cer), a pegylated phosphatidylethanolamine (PEG-PE), PEG succinate diacylglycerol (PEGS-DAG) (such as 4-0-(2',3'-di(tetradecanoyloxy)propyl-1-0-(w- methoxy(polyethoxy)ethyl) butanedioate (PEG-S-DMG)), PEG dialkoxypolypropylcarbam, N- (carbonyl-methoxypoly ethylene glycol 2000)- 1 ,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt, and those described in Table 2 of
25 WO2019051289 (incorporated by reference), and combinations of the foregoing.

 In some embodiments, sterols that can be incorporated into lipid nanoparticles include one or more of cholesterol or cholesterol derivatives, such as those in W02009/127060 or US2010/0130588, which are incorporated by reference. Additional exemplary sterols include phytosterols, including those described in Eygeris et al (2020),
30 [dx.doi.org/10.1021/acs.nanolett.0c01386](https://doi.org/10.1021/acs.nanolett.0c01386), incorporated herein by reference.

In some embodiments, the lipid particle comprises an ionizable lipid, a non-cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a sterol. The amounts of these components can be varied independently and to achieve desired properties. For example, in some embodiments, the lipid nanoparticle comprises an ionizable lipid is in an amount from
5 about 20 mol % to about 90 mol % of the total lipids (in other embodiments it may be 20-70% (mol), 30-60% (mol) or 40-50% (mol); about 50 mol % to about 90 mol % of the total lipid present in the lipid nanoparticle), a non-cationic lipid in an amount from about 5 mol % to about 30 mol % of the total lipids, a conjugated lipid in an amount from about 0.5 mol % to about 20 mol % of the total lipids, and a sterol in an amount from about 20 mol % to about 50 mol % of
10 the total lipids. The ratio of total lipid to nucleic acid (e.g., encoding the gene modifying polypeptide or template nucleic acid) can be varied as desired. For example, the total lipid to nucleic acid (mass or weight) ratio can be from about 10: 1 to about 30: 1.

In some embodiments, an ionizable lipid may be a cationic lipid, an ionizable cationic lipid, e.g., a cationic lipid that can exist in a positively charged or neutral form depending on pH,
15 or an amine-containing lipid that can be readily protonated. In some embodiments, the cationic lipid is a lipid capable of being positively charged, e.g., under physiological conditions. Exemplary cationic lipids include one or more amine group(s) which bear the positive charge. In some embodiments, the lipid particle comprises a cationic lipid in formulation with one or more of neutral lipids, ionizable amine-containing lipids, biodegradable alkyn lipids, steroids,
20 phospholipids including polyunsaturated lipids, structural lipids (e.g., sterols), PEG, cholesterol and polymer conjugated lipids. In some embodiments, the cationic lipid may be an ionizable cationic lipid. An exemplary cationic lipid as disclosed herein may have an effective pKa over 6.0. In embodiments, a lipid nanoparticle may comprise a second cationic lipid having a different effective pKa (e.g., greater than the first effective pKa), than the first cationic lipid. A lipid
25 nanoparticle may comprise between 40 and 60 mol percent of a cationic lipid, a neutral lipid, a steroid, a polymer conjugated lipid, and a therapeutic agent, e.g., a nucleic acid (e.g., RNA) described herein (e.g., a template nucleic acid or a nucleic acid encoding a gene modifying polypeptide), encapsulated within or associated with the lipid nanoparticle. In some embodiments, the nucleic acid is co-formulated with the cationic lipid. The nucleic acid may be
30 adsorbed to the surface of an LNP, e.g., an LNP comprising a cationic lipid. In some embodiments, the nucleic acid may be encapsulated in an LNP, e.g., an LNP comprising a

cationic lipid. In some embodiments, the lipid nanoparticle may comprise a targeting moiety, e.g., coated with a targeting agent. In embodiments, the LNP formulation is biodegradable. In some embodiments, a lipid nanoparticle comprising one or more lipid described herein, e.g., Formula (i), (ii), (ii), (vii) and/or (ix) encapsulates at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98% or 100% of an RNA molecule, e.g., template RNA and/or a mRNA encoding the gene modifying polypeptide.

In some embodiments, the lipid to nucleic acid ratio (mass/mass ratio; w/w ratio) can be in the range of from about 1 : 1 to about 25: 1, from about 10: 1 to about 14: 1, from about 3 : 1 to about 15: 1, from about 4: 1 to about 10: 1, from about 5: 1 to about 9: 1, or about 6: 1 to about 9: 1. The amounts of lipids and nucleic acid can be adjusted to provide a desired N/P ratio, for example, N/P ratio of 3, 4, 5, 6, 7, 8, 9, 10 or higher. Generally, the lipid nanoparticle formulation's overall lipid content can range from about 5 mg/ml to about 30 mg/mL.

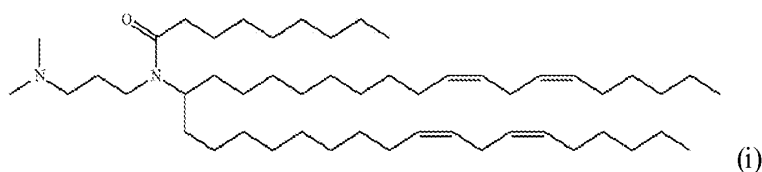
Exemplary ionizable lipids that can be used in lipid nanoparticle formulations include, without limitation, those listed in Table 1 of WO2019051289, incorporated herein by reference. Additional exemplary lipids include, without limitation, one or more of the following formulae: X of US2016/0311759; I of US20150376115 or in US2016/0376224; I, II or III of US20160151284; I, IA, II, or IIA of US20170210967; I-c of US20150140070; A of US2013/0178541; I of US2013/0303587 or US2013/0123338; I of US2015/0141678; II, III, IV, or V of US2015/0239926; I of US2017/0119904; I or II of WO2017/117528; A of US2012/0149894; A of US2015/0057373; A of WO2013/116126; A of US2013/0090372; A of US2013/0274523; A of US2013/0274504; A of US2013/0053572; A of W02013/016058; A of W02012/162210; I of US2008/042973; I, II, III, or IV of US2012/01287670; I or II of US2014/0200257; I, II, or III of US2015/0203446; I or III of US2015/0005363; I, IA, IB, IC, ID, II, IIA, IIB, IIC, IID, or III-XXIV of US2014/0308304; of US2013/0338210; I, II, III, or IV of W02009/132131; A of US2012/01011478; I or XXXV of US2012/0027796; XIV or XVII of US2012/0058144; of US2013/0323269; I of US2011/0117125; I, II, or III of US2011/0256175; I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII of US2012/0202871; I, II, III, IV, V, VI, VII, VIII, X, XII, XIII, XIV, XV, or XVI of US2011/0076335; I or II of US2006/008378; I of US2013/0123338; I or X-A-Y-Z of US2015/0064242; XVI, XVII, or XVIII of US2013/0022649; I, II, or III of US2013/0116307; I, II, or III of US2013/0116307; I or II of

US2010/0062967; I-X of US2013/0189351; I of US2014/0039032; V of US2018/0028664; I of US2016/0317458; I of US2013/0195920; 5, 6, or 10 of US10,221,127; III-3 of WO2018/081480; I-5 or I-8 of WO2020/081938; 18 or 25 of US9,867,888; A of US2019/0136231; II of WO2020/219876; 1 of US2012/0027803; OF-02 of US2019/0240349; 23 of US10,086,013; cKK-E12/A6 of Miao et al (2020); C12-200 of WO2010/053572; 7C1 of Dahlman et al (2017); 304-O13 or 503-O13 of Whitehead et al; TS-P4C2 of US9,708,628; I of WO2020/106946; I of WO2020/106946.

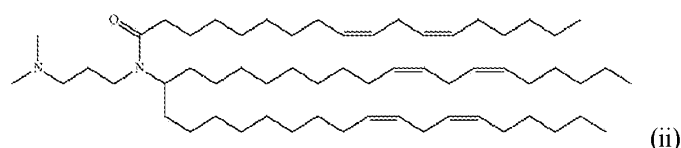
In some embodiments, the ionizable lipid is MC3 (6Z,9Z,28Z,3 lZ)-heptatriaconta-6,9,28,3 l-tetraen-19-yl-4-(dimethylamino) butanoate (DLin-MC3-DMA or MC3), e.g., as described in Example 9 of WO2019051289A9 (incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is the lipid ATX-002, e.g., as described in Example 10 of WO2019051289A9 (incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is (13Z,16Z)-A,A-dimethyl-3- nonyldocosa-13, 16-dien-1-amine (Compound 32), e.g., as described in Example 11 of WO2019051289A9 (incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is Compound 6 or Compound 22, e.g., as described in Example 12 of WO2019051289A9 (incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate (SM-102); e.g., as described in Example 1 of US9,867,888(incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is 9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate (LP01) e.g., as synthesized in Example 13 of WO2015/095340(incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is Di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), e.g. as synthesized in Example 7, 8, or 9 of US2012/0027803(incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is 1,1'-((2-(4-(2-((2-(Bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethyl)azanediyl)bis(dodecan-2-ol) (C12-200), e.g., as synthesized in Examples 14 and 16 of WO2010/053572(incorporated by reference herein in its entirety). In some embodiments, the ionizable lipid is; Imidazole cholesterol ester (ICE) lipid (3S, 10R, 13R, 17R)-10, 13-dimethyl-17- ((R)-6-methylheptan-2-yl)-2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16,

17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3-(1H-imidazol-4-yl)propanoate, e.g.,
Structure (I) from WO2020/106946 (incorporated by reference herein in its entirety).

Some non-limiting examples of lipid compounds that may be used (e.g., in combination
with other lipid components) to form lipid nanoparticles for the delivery of compositions
described herein, e.g., nucleic acid (e.g., RNA) described herein (e.g., a template nucleic acid or
5 a nucleic acid encoding a gene modifying polypeptide) includes,

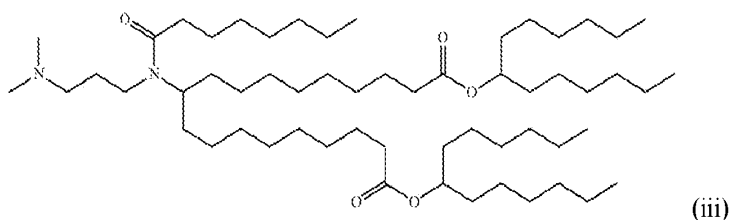


In some embodiments an LNP comprising Formula (i) is used to deliver a gene
modifying composition described herein to the liver and/or hepatocyte cells.

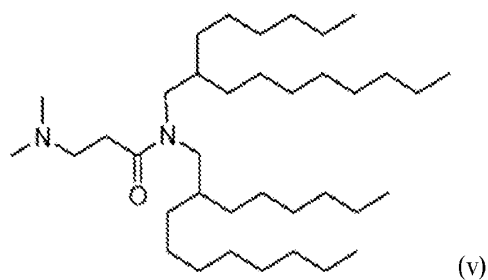
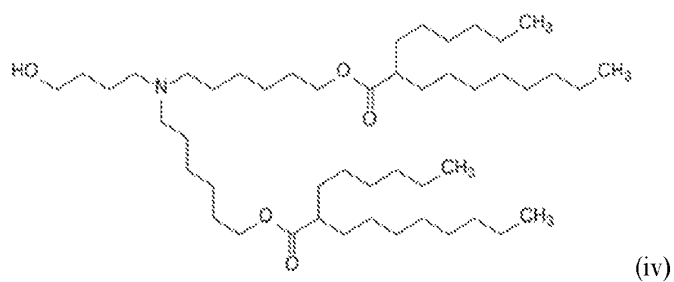


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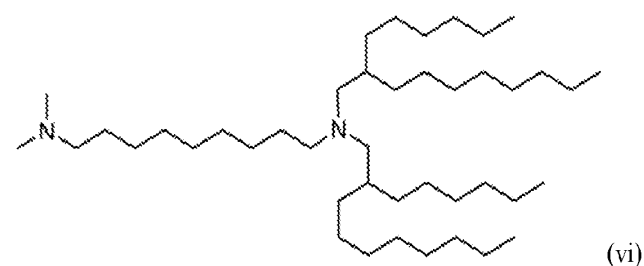
In some embodiments an LNP comprising Formula (ii) is used to deliver a gene
modifying composition described herein to the liver and/or hepatocyte cells.



In some embodiments an LNP comprising Formula (iii) is used to deliver a gene
15 modifying composition described herein to the liver and/or hepatocyte cells.

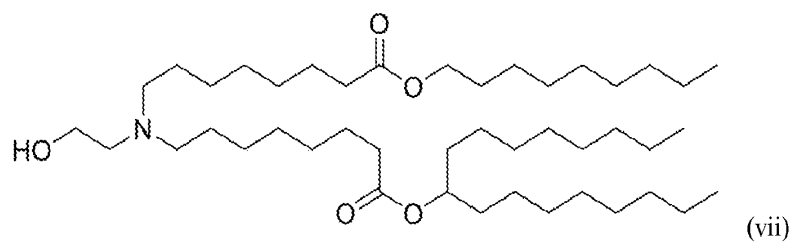


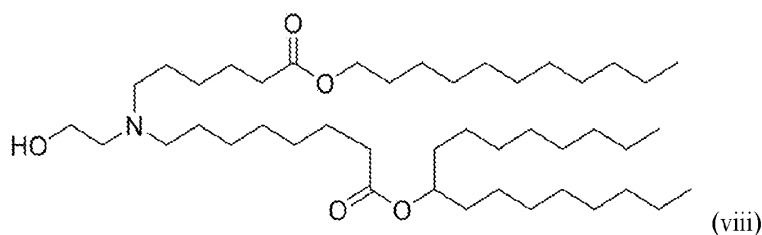
In some embodiments an LNP comprising Formula (v) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.



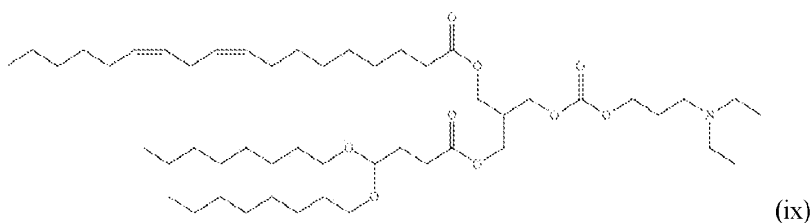
5

In some embodiments an LNP comprising Formula (vi) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.

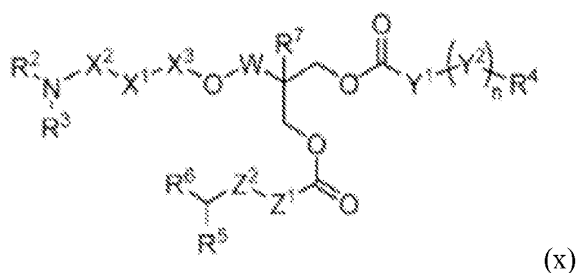




In some embodiments an LNP comprising Formula (viii) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.

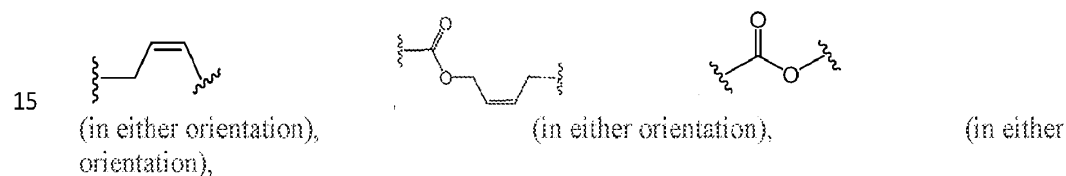


- 5 In some embodiments an LNP comprising Formula (ix) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.

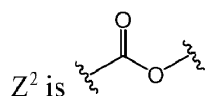


wherein

- 10 X^1 is O, NR^1 , or a direct bond, X^2 is C2-5 alkylene, X^3 is $C(=O)$ or a direct bond, R^1 is H or Me, R^3 is C1-3 alkyl, R^2 is C1-3 alkyl, or R^2 taken together with the nitrogen atom to which it is attached and 1-3 carbon atoms of X^2 form a 4-, 5-, or 6-membered ring, or X^1 is NR^1 , R^1 and R^2 taken together with the nitrogen atoms to which they are attached form a 5- or 6-membered ring, or R^2 taken together with R^3 and the nitrogen atom to which they are attached form a 5-, 6-, or 7-membered ring, Y^1 is C2-12 alkylene, Y^2 is selected from

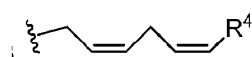


n is 0 to 3, R⁴ is C1-15 alkyl, Z¹ is C1-6 alkylene or a direct bond,



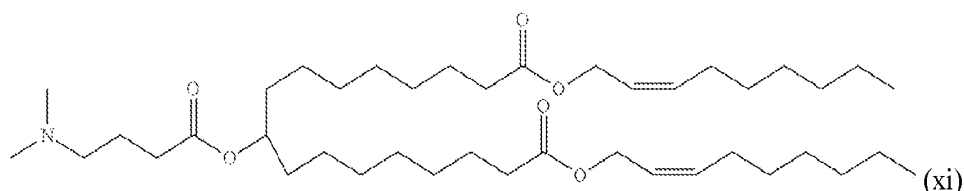
(in either orientation) or absent, provided that if Z¹ is a direct bond, Z² is absent;

- 5 R⁵ is C5-9 alkyl or C6-10 alkoxy, R⁶ is C5-9 alkyl or C6-10 alkoxy, W is methylene or a direct bond, and R⁷ is H or Me, or a salt thereof, provided that if R³ and R² are C2 alkyls, X¹ is O, X² is linear C3 alkylene, X³ is C(=O), Y¹ is linear C6 alkylene, (Y²)_n-R⁴ is



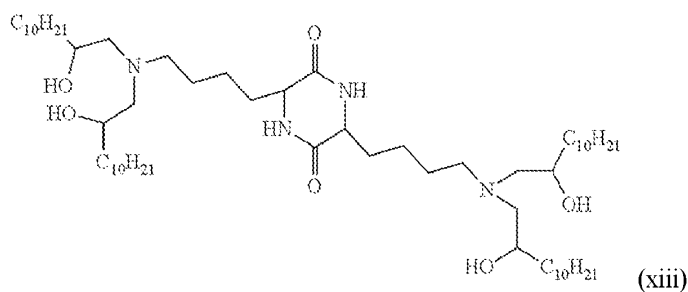
, R⁴ is linear C5 alkyl, Z¹ is C2 alkylene, Z² is absent, W is methylene, and R⁷ is H, then R⁵ and R⁶ are not C_x alkoxy.

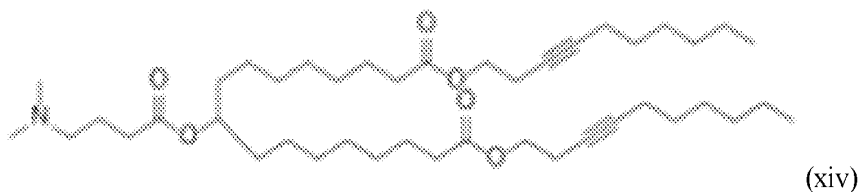
- 10 In some embodiments an LNP comprising Formula (xii) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.



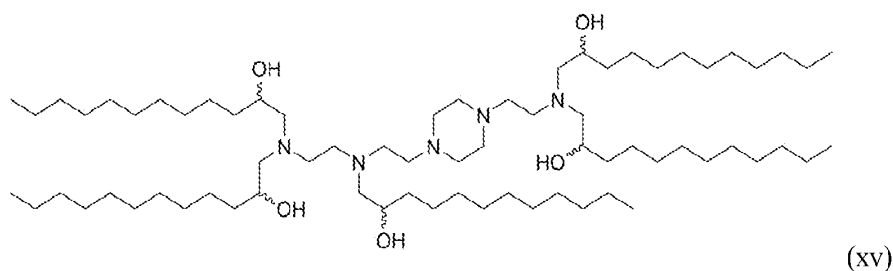
In some embodiments an LNP comprising Formula (xi) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.

- 15
- where R=
- (xii)

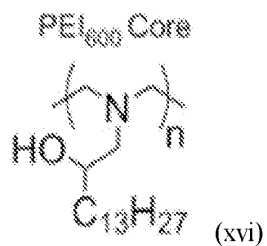




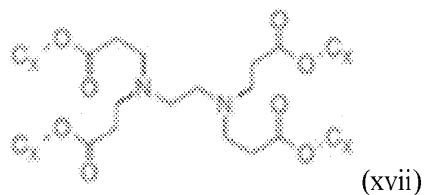
In some embodiments an LNP comprises a compound of Formula (xiii) and a compound of Formula (xiv).

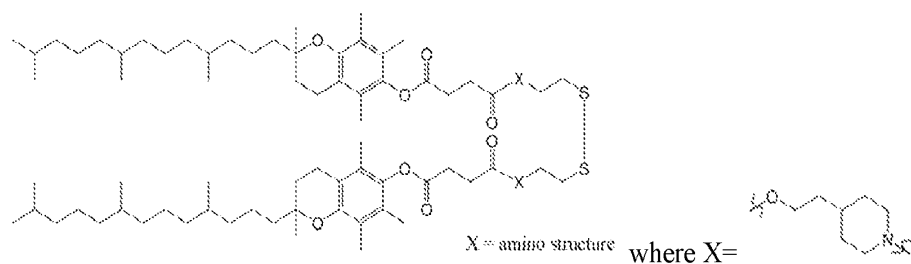


- 5 In some embodiments an LNP comprising Formula (xv) is used to deliver a gene modifying composition described herein to the liver and/or hepatocyte cells.

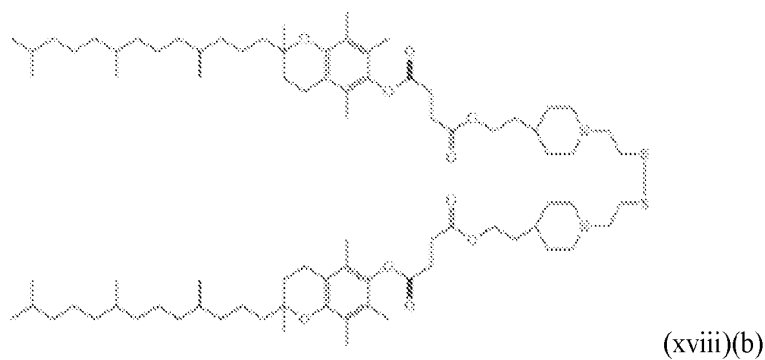


In some embodiments an LNP comprising a formulation of Formula (xvi) is used to deliver a gene modifying composition described herein to the lung endothelial cells.

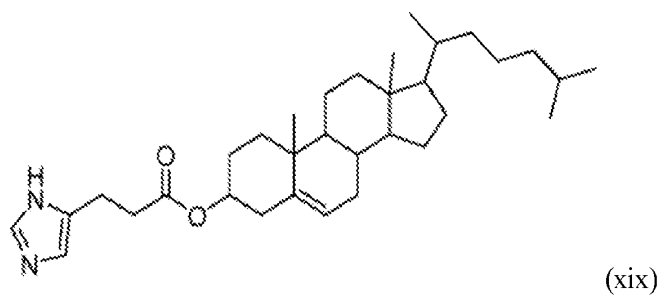




(xviii) (a)



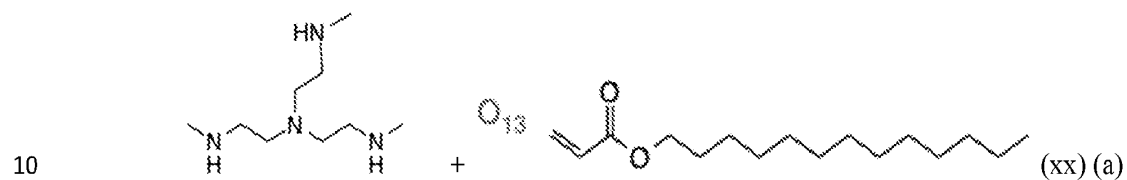
(xviii)(b)

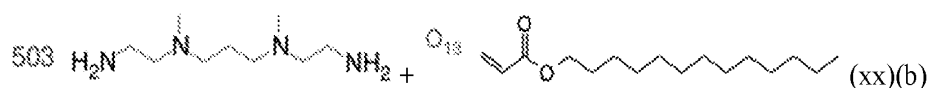


(xix)

5

In some embodiments, a lipid compound used to form lipid nanoparticles for the delivery of compositions described herein, e.g., nucleic acid (e.g., RNA) described herein (e.g., a template nucleic acid or a nucleic acid encoding a gene modifying polypeptide) is made by one of the following reactions:





Exemplary non-cationic lipids include, but are not limited to, distearoyl-sn-glycero-phosphoethanolamine, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG),

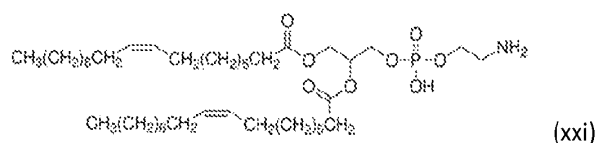
5 dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine

10 ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), monomethyl-phosphatidylethanolamine (such as 16-O-monomethyl PE), dimethyl- phosphatidylethanolamine (such as 16-O-dimethyl PE), 18-l-trans PE, 1-stearoyl-2-oleoyl- phosphatidylethanolamine (SOPE), hydrogenated soy phosphatidylcholine (HSPC), egg phosphatidylcholine (EPC), dioleoylphosphatidylserine (DOPS), sphingomyelin (SM),

15 dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), distearoylphosphatidylglycerol (DSPG), dierycylphosphatidylcholine (DEPC), palmitoyloleoylphosphatidylglycerol (POPG), dielaidoyl-phosphatidylethanolamine (DEPE), lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, egg sphingomyelin (ESM), cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, lysophosphatidylcholine,

20 dilinoleoylphosphatidylcholine, or mixtures thereof. It is understood that other diacylphosphatidylcholine and diacylphosphatidylethanolamine phospholipids can also be used. The acyl groups in these lipids are preferably acyl groups derived from fatty acids having C10-C24 carbon chains, e.g., lauroyl, myristoyl, palmitoyl, stearoyl, or oleoyl. Additional exemplary lipids, in certain embodiments, include, without limitation, those described in Kim et al. (2020)

25 [dx.doi.org/10.1021/acs.nanolett.0c01386](https://doi.org/10.1021/acs.nanolett.0c01386), incorporated herein by reference. Such lipids include, in some embodiments, plant lipids found to improve liver transfection with mRNA (e.g., DGTS). In some embodiments, the non-cationic lipid may have the following structure,



Other examples of non-cationic lipids suitable for use in the lipid nanoparticles include, without limitation, nonphosphorous lipids such as, e.g., stearylamine, dodecylamine, hexadecylamine, acetyl palmitate, glycerol ricinoleate, hexadecyl stearate, isopropyl myristate, amphoteric acrylic polymers, triethanolamine-lauryl sulfate, alkyl-aryl sulfate polyethoxylated fatty acid amides, dioctadecyl dimethyl ammonium bromide, ceramide, sphingomyelin, and the like. Other non-cationic lipids are described in WO2017/099823 or US patent publication US2018/0028664, the contents of which is incorporated herein by reference in their entirety.

In some embodiments, the non-cationic lipid is oleic acid or a compound of Formula I, II, or IV of US2018/0028664, incorporated herein by reference in its entirety. The non-cationic lipid can comprise, for example, 0-30% (mol) of the total lipid present in the lipid nanoparticle. In some embodiments, the non-cationic lipid content is 5-20% (mol) or 10-15% (mol) of the total lipid present in the lipid nanoparticle. In embodiments, the molar ratio of ionizable lipid to the neutral lipid ranges from about 2:1 to about 8:1 (e.g., about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, or 8:1).

In some embodiments, the lipid nanoparticles do not comprise any phospholipids.

In some embodiments, the lipid nanoparticle can further comprise a component, such as a sterol, to provide membrane integrity. One exemplary sterol that can be used in the lipid nanoparticle is cholesterol and derivatives thereof. Non-limiting examples of cholesterol derivatives include polar analogues such as 5 α -cholestanol, 5 β -coprostanol, choiesterolyl-(2'-hydroxy)-ethyl ether, choiesterolyl-(4'-hydroxy)-butyl ether, and 6-ketocholestanol; non-polar analogues such as 5 α -cholestane, cholestenone, 5 α -cholestanone, 5 β -cholestanone, and choiesterolyl decanoate; and mixtures thereof. In some embodiments, the cholesterol derivative is a polar analogue, e.g., choiesterolyl-(4'-hydroxy)-butyl ether. Exemplary cholesterol derivatives are described in PCT publication W02009/127060 and US patent publication US2010/0130588, each of which is incorporated herein by reference in its entirety.

In some embodiments, the component providing membrane integrity, such as a sterol, can comprise 0-50% (mol) (e.g., 0-10%, 10-20%, 20-30%, 30-40%, or 40-50%) of the total lipid

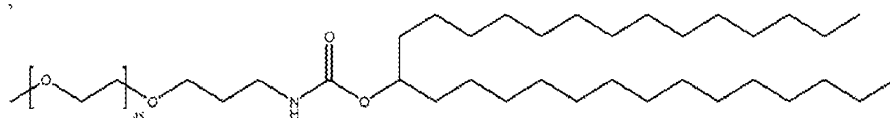
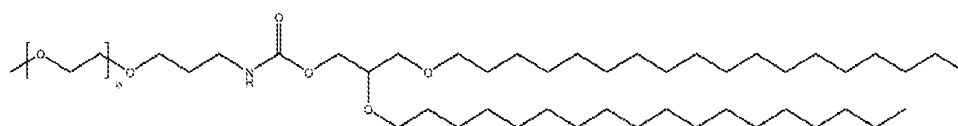
present in the lipid nanoparticle. In some embodiments, such a component is 20-50% (mol) 30-40% (mol) of the total lipid content of the lipid nanoparticle.

In some embodiments, the lipid nanoparticle can comprise a polyethylene glycol (PEG) or a conjugated lipid molecule. Generally, these are used to inhibit aggregation of lipid nanoparticles and/or provide steric stabilization. Exemplary conjugated lipids include, but are not limited to, PEG-lipid conjugates, polyoxazoline (POZ)-lipid conjugates, polyamide-lipid conjugates (such as ATTA-lipid conjugates), cationic-polymer lipid (CPL) conjugates, and mixtures thereof. In some embodiments, the conjugated lipid molecule is a PEG-lipid conjugate, for example, a (methoxy polyethylene glycol)-conjugated lipid.

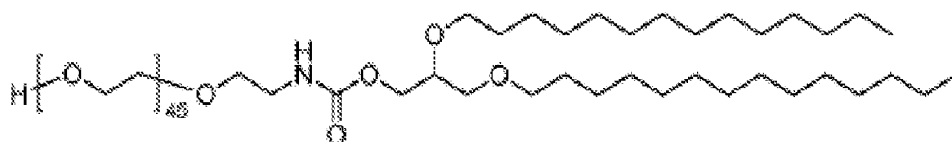
Exemplary PEG-lipid conjugates include, but are not limited to, PEG-diacylglycerol (DAG) (such as 1-(monomethoxy-polyethyleneglycol)-2,3-dimyristoylglycerol (PEG-DMG)), PEG-dialkylxypropyl (DAA), PEG-phospholipid, PEG-ceramide (Cer), a pegylated phosphatidylethanolamine (PEG-PE), 1,2-dimyristoyl-sn-glycerol, methoxypoly ethylene glycol (DMG-PEG-2K), PEG succinate diacylglycerol (PEGs-DAG) (such as 4-0-(2',3'-di(tetradecanoyloxy)propyl-1-0-(w-methoxy(polyethoxy)ethyl) butanedioate (PEG-S-DMG)), PEG dialkoxypopylcarbam, N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt, or a mixture thereof. Additional exemplary PEG-lipid conjugates are described, for example, in US5,885,613, US6,287,591, US2003/0077829, US2003/0077829, US2005/0175682, US2008/0020058, US2011/0117125, US2010/0130588, US2016/0376224, US2017/0119904, and US/099823, the contents of all of which are incorporated herein by reference in their entirety. In some embodiments, a PEG-lipid is a compound of Formula III, III-a-1, III-a-2, III-b-1, III-b-2, or V of US2018/0028664, the content of which is incorporated herein by reference in its entirety. In some embodiments, a PEG-lipid is of Formula II of US20150376115 or US2016/0376224, the content of both of which is incorporated herein by reference in its entirety. In some embodiments, the PEG-DAA conjugate can be, for example, PEG-dilauryloxypropyl, PEG- dimyristyloxypropyl, PEG-dipalmityloxypropyl, or PEG-distearyloxypropyl. The PEG-lipid can be one or more of PEG-DMG, PEG-dilaurylglycerol, PEG-dipalmitoylglycerol, PEG- disteryllycerol, PEG-dilaurylglycamide, PEG-dimyristylglycamide, PEG- dipalmitoylglycamide, PEG-disterylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl] carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG- DMB (3,4-

Ditetradecoxybenzyl- [omega]-methyl-poly(ethylene glycol) ether), and 1,2- dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]. In some embodiments, the PEG-lipid comprises PEG-DMG, 1,2- dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]. In some embodiments, the PEG-lipid comprises a

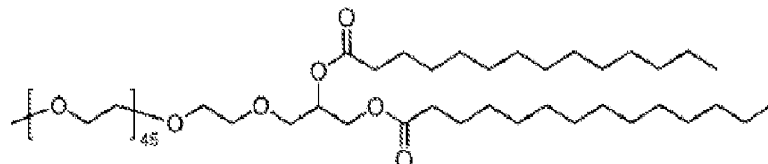
5 structure selected from:



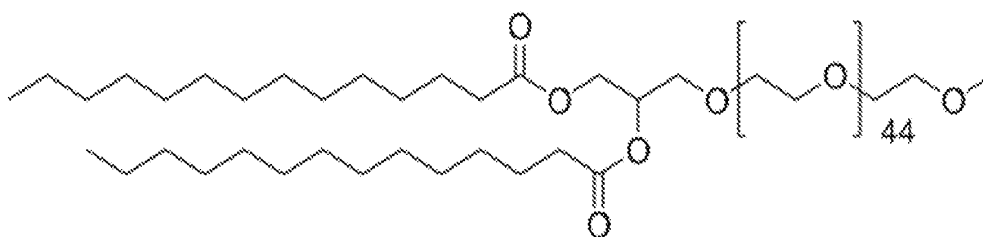
(xxii),



(xxiii),



(xxiv), and



(xxv).

In some embodiments, lipids conjugated with a molecule other than a PEG can also be used in place of PEG-lipid. For example, polyoxazoline (POZ)-lipid conjugates, polyamide-lipid conjugates (such as ATTA-lipid conjugates), and cationic-polymer lipid (GPL) conjugates can be used in place of or in addition to the PEG-lipid.

Exemplary conjugated lipids, i.e., PEG-lipids, (POZ)-lipid conjugates, ATTA-lipid conjugates and cationic polymer-lipids are described in the PCT and LIS patent applications listed in Table 2 of WO2019051289A9 and in WO2020106946A1, the contents of all of which are incorporated herein by reference in their entirety.

5 In some embodiments an LNP comprises a compound of Formula (xix), a compound of Formula (xxi) and a compound of Formula (xxv). In some embodiments an LNP comprising a formulation of Formula (xix), Formula (xxi) and Formula (xxv) is used to deliver a gene modifying composition described herein to the lung or pulmonary cells.

10 In some embodiments, a lipid nanoparticle may comprise one or more cationic lipids selected from Formula (i), Formula (ii), Formula (iii), Formula (vii), and Formula (ix). In some embodiments, the LNP may further comprise one or more neutral lipid, e.g., DSPC, DPPC, DMPC, DOPC, POPC, DOPE, SM, a steroid, e.g., cholesterol, and/or one or more polymer conjugated lipid, e.g., a pegylated lipid, e.g., PEG-DAG, PEG-PE, PEG-S-DAG, PEG-cer or a PEG dialkoxypropylcarbamate.

15 In some embodiments, the PEG or the conjugated lipid can comprise 0-20% (mol) of the total lipid present in the lipid nanoparticle. In some embodiments, PEG or the conjugated lipid content is 0.5- 10% or 2-5% (mol) of the total lipid present in the lipid nanoparticle. Molar ratios of the ionizable lipid, non-cationic-lipid, sterol, and PEG/conjugated lipid can be varied as needed. For example, the lipid particle can comprise 30-70% ionizable lipid by mole or by total weight of the composition, 0-60% cholesterol by mole or by total weight of the composition, 0-30% non-cationic-lipid by mole or by total weight of the composition and 1-10% conjugated lipid by mole or by total weight of the composition. Preferably, the composition comprises 30-40% ionizable lipid by mole or by total weight of the composition, 40-50% cholesterol by mole or by total weight of the composition, and 10- 20% non-cationic-lipid by mole or by total weight of the composition. In some other embodiments, the composition is 50-75% ionizable lipid by mole or by total weight of the composition, 20-40% cholesterol by mole or by total weight of the composition, and 5 to 10% non-cationic-lipid, by mole or by total weight of the composition and 1-10% conjugated lipid by mole or by total weight of the composition. The composition may contain 60-70% ionizable lipid by mole or by total weight of the composition, 25-35% cholesterol by mole or by total weight of the composition, and 5-10% non-cationic-lipid by mole

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or by total weight of the composition. The composition may also contain up to 90% ionizable lipid by mole or by total weight of the composition and 2 to 15% non-cationic lipid by mole or by total weight of the composition. The formulation may also be a lipid nanoparticle formulation, for example comprising 8-30% ionizable lipid by mole or by total weight of the composition, 5-30% non-cationic lipid by mole or by total weight of the composition, and 0-20% cholesterol by mole or by total weight of the composition; 4-25% ionizable lipid by mole or by total weight of the composition, 4-25% non-cationic lipid by mole or by total weight of the composition, 2 to 25% cholesterol by mole or by total weight of the composition, 10 to 35% conjugate lipid by mole or by total weight of the composition, and 5% cholesterol by mole or by total weight of the composition; or 2-30% ionizable lipid by mole or by total weight of the composition, 2-30% non-cationic lipid by mole or by total weight of the composition, 1 to 15% cholesterol by mole or by total weight of the composition, 2 to 35% conjugate lipid by mole or by total weight of the composition, and 1-20% cholesterol by mole or by total weight of the composition; or even up to 90% ionizable lipid by mole or by total weight of the composition and 2-10% non-cationic lipids by mole or by total weight of the composition, or even 100% cationic lipid by mole or by total weight of the composition. In some embodiments, the lipid particle formulation comprises ionizable lipid, phospholipid, cholesterol and a PEG-ylated lipid in a molar ratio of 50: 10:38.5: 1.5. In some other embodiments, the lipid particle formulation comprises ionizable lipid, cholesterol and a PEG-ylated lipid in a molar ratio of 60:38.5: 1.5.

In some embodiments, the lipid particle comprises ionizable lipid, non-cationic lipid (e.g. phospholipid), a sterol (e.g., cholesterol) and a PEG-ylated lipid, where the molar ratio of lipids ranges from 20 to 70 mole percent for the ionizable lipid, with a target of 40-60, the mole percent of non-cationic lipid ranges from 0 to 30, with a target of 0 to 15, the mole percent of sterol ranges from 20 to 70, with a target of 30 to 50, and the mole percent of PEG-ylated lipid ranges from 1 to 6, with a target of 2 to 5.

In some embodiments, the lipid particle comprises ionizable lipid / non-cationic- lipid / sterol / conjugated lipid at a molar ratio of 50: 10:38.5: 1.5.

In an aspect, the disclosure provides a lipid nanoparticle formulation comprising phospholipids, lecithin, phosphatidylcholine and phosphatidylethanolamine.

In some embodiments, one or more additional compounds can also be included. Those compounds can be administered separately or the additional compounds can be included in the

lipid nanoparticles of the invention. In other words, the lipid nanoparticles can contain other compounds in addition to the nucleic acid or at least a second nucleic acid, different than the first. Without limitations, other additional compounds can be selected from the group consisting of small or large organic or inorganic molecules, monosaccharides, disaccharides, trisaccharides, oligosaccharides, polysaccharides, peptides, proteins, peptide analogs and derivatives thereof, peptidomimetics, nucleic acids, nucleic acid analogs and derivatives, an extract made from biological materials, or any combinations thereof.

In some embodiments, a lipid nanoparticle (or a formulation comprising lipid nanoparticles) lacks reactive impurities (e.g., aldehydes or ketones), or comprises less than a preselected level of reactive impurities (e.g., aldehydes or ketones). While not wishing to be bound by theory, in some embodiments, a lipid reagent is used to make a lipid nanoparticle formulation, and the lipid reagent may comprise a contaminating reactive impurity (e.g., an aldehyde or ketone). A lipid reagent may be selected for manufacturing based on having less than a preselected level of reactive impurities (e.g., aldehydes or ketones). Without wishing to be bound by theory, in some embodiments, aldehydes can cause modification and damage of RNA, e.g., cross-linking between bases and/or covalently conjugating lipid to RNA (e.g., forming lipid-RNA adducts). This may, in some instances, lead to failure of a reverse transcriptase reaction and/or incorporation of inappropriate bases, e.g., at the site(s) of lesion(s), e.g., a mutation in a newly synthesized target DNA.

In some embodiments, a lipid nanoparticle formulation is produced using a lipid reagent comprising less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content. In some embodiments, a lipid nanoparticle formulation is produced using a lipid reagent comprising less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species. In some embodiments, a lipid nanoparticle formulation is produced using a lipid reagent comprising: (i) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content; and (ii) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species. In some embodiments, the lipid nanoparticle formulation is produced using a plurality of lipid reagents, and each lipid reagent of the plurality

independently meets one or more criterion described in this paragraph. In some embodiments, each lipid reagent of the plurality meets the same criterion, e.g., a criterion of this paragraph.

In some embodiments, the lipid nanoparticle formulation comprises less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content. In some embodiments, the lipid nanoparticle formulation comprises less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species. In some embodiments, the lipid nanoparticle formulation comprises: (i) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content; and (ii) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species.

In some embodiments, one or more, or optionally all, of the lipid reagents used for a lipid nanoparticle as described herein or a formulation thereof comprise less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content. In some embodiments, one or more, or optionally all, of the lipid reagents used for a lipid nanoparticle as described herein or a formulation thereof comprise less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species. In some embodiments, one or more, or optionally all, of the lipid reagents used for a lipid nanoparticle as described herein or a formulation thereof comprise: (i) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% total reactive impurity (e.g., aldehyde) content; and (ii) less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of any single reactive impurity (e.g., aldehyde) species.

In some embodiments, total aldehyde content and/or quantity of any single reactive impurity (e.g., aldehyde) species is determined by liquid chromatography (LC), e.g., coupled with tandem mass spectrometry (MS/MS), e.g., according to the method described in Example 40 of PCT/US21/20948. In some embodiments, reactive impurity (e.g., aldehyde) content and/or quantity of reactive impurity (e.g., aldehyde) species is determined by detecting one or more chemical modifications of a nucleic acid molecule (e.g., an RNA molecule, e.g., as described herein) associated with the presence of reactive impurities (e.g., aldehydes), e.g., in the lipid reagents. In some embodiments, reactive impurity (e.g., aldehyde) content and/or quantity of

reactive impurity (e.g., aldehyde) species is determined by detecting one or more chemical modifications of a nucleotide or nucleoside (e.g., a ribonucleotide or ribonucleoside, e.g., comprised in or isolated from a template nucleic acid, e.g., as described herein) associated with the presence of reactive impurities (e.g., aldehydes), e.g., in the lipid reagents, e.g., according to the method described in Example 41 of PCT/US21/20948. In embodiments, chemical
5 modifications of a nucleic acid molecule, nucleotide, or nucleoside are detected by determining the presence of one or more modified nucleotides or nucleosides, e.g., using LC-MS/MS analysis, e.g., according to the method described in Example 41 of PCT/US21/20948.

In some embodiments, a nucleic acid (e.g., RNA) described herein (e.g., a template
10 nucleic acid or a nucleic acid encoding a gene modifying polypeptide) does not comprise an aldehyde modification, or comprises less than a preselected amount of aldehyde modifications. In some embodiments, on average, a nucleic acid has less than 50, 20, 10, 5, 2, or 1 aldehyde modifications per 1000 nucleotides, e.g., wherein a single cross-linking of two nucleotides is a single aldehyde modification. In some embodiments, the aldehyde modification is an RNA
15 adduct (e.g., a lipid-RNA adduct). In some embodiments, the aldehyde-modified nucleotide is cross-linking between bases. In some embodiments, a nucleic acid (e.g., RNA) described herein comprises less than 50, 20, 10, 5, 2, or 1 cross-links between nucleotide.

In some embodiments, LNPs are directed to specific tissues by the addition of targeting domains. For example, biological ligands may be displayed on the surface of LNPs to enhance
20 interaction with cells displaying cognate receptors, thus driving association with and cargo delivery to tissues wherein cells express the receptor. In some embodiments, the biological ligand may be a ligand that drives delivery to the liver, e.g., LNPs that display GalNAc result in delivery of nucleic acid cargo to hepatocytes that display asialoglycoprotein receptor (ASGPR). The work of Akinc et al. *Mol Ther* 18(7):1357-1364 (2010) teaches the conjugation of a trivalent
25 GalNAc ligand to a PEG-lipid (GalNAc-PEG-DSG) to yield LNPs dependent on ASGPR for observable LNP cargo effect (see, e.g., Figure 6 therein). Other ligand-displaying LNP formulations, e.g., incorporating folate, transferrin, or antibodies, are discussed in WO2017223135, which is incorporated herein by reference in its entirety, in addition to the references used therein, namely Kolhatkar et al., *Curr Drug Discov Technol.* 2011 8:197-206;
30 Musacchio and Torchilin, *Front Biosci.* 2011 16:1388-1412; Yu et al., *Mol Membr Biol.* 2010 27:286-298; Patil et al., *Crit Rev Ther Drug Carrier Syst.* 2008 25:1-61; Benoit et al.,

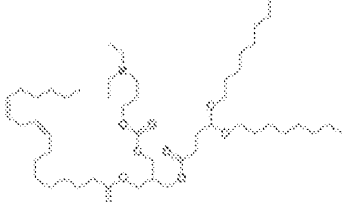
Biomacromolecules. 2011 12:2708-2714; Zhao et al., Expert Opin Drug Deliv. 2008 5:309-319; Akinc et al., Mol Ther. 2010 18:1357-1364; Srinivasan et al., Methods Mol Biol. 2012 820:105-116; Ben-Arie et al., Methods Mol Biol. 2012 757:497-507; Peer 2010 J Control Release. 20:63-68; Peer et al., Proc Natl Acad Sci U S A. 2007 104:4095-4100; Kim et al., Methods Mol Biol. 2011 721:339-353; Subramanya et al., Mol Ther. 2010 18:2028-2037; Song et al., Nat Biotechnol. 2005 23:709-717; Peer et al., Science. 2008 319:627-630; and Peer and Lieberman, Gene Ther. 2011 18:1127-1133.

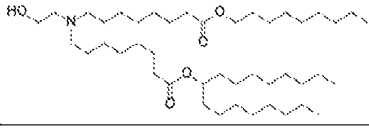
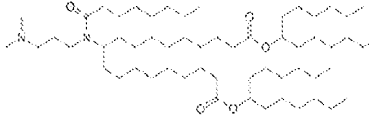
In some embodiments, LNPs are selected for tissue-specific activity by the addition of a Selective ORgan Targeting (SORT) molecule to a formulation comprising traditional components, such as ionizable cationic lipids, amphipathic phospholipids, cholesterol and poly(ethylene glycol) (PEG) lipids. The teachings of Cheng et al. Nat Nanotechnol 15(4):313-320 (2020) demonstrate that the addition of a supplemental “SORT” component precisely alters the *in vivo* RNA delivery profile and mediates tissue-specific (e.g., lungs, liver, spleen) gene delivery and editing as a function of the percentage and biophysical property of the SORT molecule.

In some embodiments, the LNPs comprise biodegradable, ionizable lipids. In some embodiments, the LNPs comprise (9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9,12-dienoate, also called 3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl (9Z,12Z)-octadeca-9,12-dienoate) or another ionizable lipid. See, e.g, lipids of WO2019/067992, WO/2017/173054, WO2015/095340, and WO2014/136086, as well as references provided therein. In some embodiments, the term cationic and ionizable in the context of LNP lipids is interchangeable, e.g., wherein ionizable lipids are cationic depending on the pH.

In some embodiments, an LNP described herein comprises a lipid described in Table 19

Table 19 Exemplary lipids

LIPID ID	Chemical Name	Molecular Weight	Structure
LIPIDV003	(9Z,12Z)-3-((4,4-bis(octyloxy)butanoyl)oxy)-2-(((3-(diethylamino)propoxy)carbonyl)oxy)methyl)propyl octadeca-9, 12-dienoate	852.29	

LIPID ID	Chemical Name	Molecular Weight	Structure
LIPIDV004	Heptadecan-9-yl 8-((2-hydroxyethyl)(3-(nonyloxy)-8-oxooctyl)amino)octanoate	710.18	
LIPIDV005		919.56	

In some embodiments, multiple components of a gene modifying system may be prepared as a single LNP formulation, e.g., an LNP formulation comprises mRNA encoding for the gene modifying polypeptide and an RNA template. Ratios of nucleic acid components may be varied in order to maximize the properties of a therapeutic. In some embodiments, the ratio of RNA template to mRNA encoding a gene modifying polypeptide is about 1:1 to 100:1, e.g., about 1:1 to 20:1, about 20:1 to 40:1, about 40:1 to 60:1, about 60:1 to 80:1, or about 80:1 to 100:1, by molar ratio. In other embodiments, a system of multiple nucleic acids may be prepared by separate formulations, e.g., one LNP formulation comprising a template RNA and a second LNP formulation comprising an mRNA encoding a gene modifying polypeptide. In some embodiments, the system may comprise more than two nucleic acid components formulated into LNPs. In some embodiments, the system may comprise a protein, e.g., a gene modifying polypeptide, and a template RNA formulated into at least one LNP formulation.

In some embodiments, the average LNP diameter of the LNP formulation may be between 10s of nm and 100s of nm, e.g., measured by dynamic light scattering (DLS). In some embodiments, the average LNP diameter of the LNP formulation may be from about 40 nm to about 150 nm, such as about 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, or 150 nm. In some embodiments, the average LNP diameter of the LNP formulation may be from about 50 nm to about 100 nm, from about 50 nm to about 90 nm, from about 50 nm to about 80 nm, from about 50 nm to about 70 nm, from about 50 nm to about 60 nm, from about 60 nm to about 100 nm, from about 60 nm to about 90 nm, from about 60 nm to about 80 nm, from about 60 nm to about 70 nm, from about 70 nm to about 100 nm, from about 70 nm to about 90 nm, from about 70 nm to about 80 nm, from about 80 nm to about 100 nm, from about 80 nm to about 90 nm, or from about 90 nm to about 100 nm. In some embodiments,

the average LNP diameter of the LNP formulation may be from about 70 nm to about 100 nm. In a particular embodiment, the average LNP diameter of the LNP formulation may be about 80 nm. In some embodiments, the average LNP diameter of the LNP formulation may be about 100 nm. In some embodiments, the average LNP diameter of the LNP formulation ranges from
5 about 1 mm to about 500 mm, from about 5 mm to about 200 mm, from about 10 mm to about 100 mm, from about 20 mm to about 80 mm, from about 25 mm to about 60 mm, from about 30 mm to about 55 mm, from about 35 mm to about 50 mm, or from about 38 mm to about 42 mm.

An LNP may, in some instances, be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of an LNP, e.g., the particle size distribution of the lipid
10 nanoparticles. A small (e.g., less than 0.3) polydispersity index generally indicates a narrow particle size distribution. An LNP may have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some embodiments, the polydispersity index of an LNP may be from about 0.10 to about 0.20.

15 The zeta potential of an LNP may be used to indicate the electrokinetic potential of the composition. In some embodiments, the zeta potential may describe the surface charge of an LNP. Lipid nanoparticles with relatively low charges, positive or negative, are generally desirable, as more highly charged species may interact undesirably with cells, tissues, and other elements in the body. In some embodiments, the zeta potential of an LNP may be from about -10
20 mV to about +20 mV, from about -10 mV to about +15 mV, from about -10 mV to about +10 mV, from about -10 mV to about +5 mV, from about -10 mV to about 0 mV, from about -10 mV to about -5 mV, from about -5 mV to about +20 mV, from about -5 mV to about +15 mV, from about -5 mV to about +10 mV, from about -5 mV to about +5 mV, from about -5 mV to about 0 mV, from about 0 mV to about +20 mV, from about 0 mV to about +15 mV, from about 0 mV to
25 about +10 mV, from about 0 mV to about +5 mV, from about +5 mV to about +20 mV, from about +5 mV to about +15 mV, or from about +5 mV to about +10 mV.

The efficiency of encapsulation of a protein and/or nucleic acid, e.g., gene modifying polypeptide or mRNA encoding the polypeptide, describes the amount of protein and/or nucleic acid that is encapsulated or otherwise associated with an LNP after preparation, relative to the
30 initial amount provided. The encapsulation efficiency is desirably high (e.g., close to 100%). The encapsulation efficiency may be measured, for example, by comparing the amount of protein or

nucleic acid in a solution containing the lipid nanoparticle before and after breaking up the lipid nanoparticle with one or more organic solvents or detergents. An anion exchange resin may be used to measure the amount of free protein or nucleic acid (e.g., RNA) in a solution.

Fluorescence may be used to measure the amount of free protein and/or nucleic acid (e.g., RNA)

5 in a solution. For the lipid nanoparticles described herein, the encapsulation efficiency of a protein and/or nucleic acid may be at least 50%, for example 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%. In some embodiments, the encapsulation efficiency may be at least 80%. In some embodiments, the encapsulation efficiency may be at least 90%. In some embodiments, the encapsulation
10 efficiency may be at least 95%.

An LNP may optionally comprise one or more coatings. In some embodiments, an LNP may be formulated in a capsule, film, or tablet having a coating. A capsule, film, or tablet including a composition described herein may have any useful size, tensile strength, hardness or density.

15 Additional exemplary lipids, formulations, methods, and characterization of LNPs are taught by WO2020061457, which is incorporated herein by reference in its entirety.

In some embodiments, *in vitro* or *ex vivo* cell lipofections are performed using Lipofectamine MessengerMax (Thermo Fisher) or TransIT-mRNA Transfection Reagent (Mirus Bio). In certain embodiments, LNPs are formulated using the GenVoy_ILM ionizable lipid mix
20 (Precision NanoSystems). In certain embodiments, LNPs are formulated using 2,2-dilinoyleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA) or dilinoylelmethyl-4-dimethylaminobutyrate (DLin-MC3-DMA or MC3), the formulation and *in vivo* use of which are taught in Jayaraman et al. Angew Chem Int Ed Engl 51(34):8529-8533 (2012), incorporated herein by reference in its entirety.

25 LNP formulations optimized for the delivery of CRISPR-Cas systems, e.g., Cas9-gRNA RNP, gRNA, Cas9 mRNA, are described in WO2019067992 and WO2019067910, both incorporated by reference.

Additional specific LNP formulations useful for delivery of nucleic acids are described in US8158601 and US8168775, both incorporated by reference, which include formulations used in
30 patisiran, sold under the name ONPATPRO.

Exemplary dosing of gene modifying LNP may include about 0.1, 0.25, 0.3, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, or 100 mg/kg (RNA). Exemplary dosing of AAV comprising a nucleic acid encoding one or more components of the system may include an MOI of about 10^{11} , 10^{12} , 10^{13} , and 10^{14} vg/kg.

5 ***Kits, Articles of Manufacture, and Pharmaceutical Compositions***

In an aspect the disclosure provides a kit comprising a gene modifying polypeptide or a gene modifying system, e.g., as described herein. In some embodiments, the kit comprises a gene modifying polypeptide (or a nucleic acid encoding the polypeptide) and a template RNA (or DNA encoding the template RNA). In some embodiments, the kit further comprises a reagent
10 for introducing the system into a cell, e.g., transfection reagent, LNP, and the like. In some embodiments, the kit is suitable for any of the methods described herein. In some embodiments, the kit comprises one or more elements, compositions (e.g., pharmaceutical compositions), gene modifying polypeptides, and/or gene modifying systems, or a functional fragment or component thereof, e.g., disposed in an article of manufacture. In some embodiments, the kit comprises
15 instructions for use thereof.

In an aspect, the disclosure provides an article of manufacture, e.g., in which a kit as described herein, or a component thereof, is disposed.

In an aspect, the disclosure provides a pharmaceutical composition comprising a gene modifying polypeptide or a gene modifying system, e.g., as described herein. In some
20 embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient. In some embodiments, the pharmaceutical composition comprises a template RNA and/or an RNA encoding the polypeptide. In embodiments, the pharmaceutical composition has one or more (e.g., 1, 2, 3, or 4) of the following characteristics:

- (a) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) DNA template relative
25 to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;
- (b) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) uncapped RNA relative to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;
- (c) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) partial length RNAs relative to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;
- 30 (d) substantially lacks unreacted cap dinucleotides.

Chemistry, Manufacturing, and Controls (CMC)

Purification of protein therapeutics is described, for example, in Franks, *Protein Biotechnology: Isolation, Characterization, and Stabilization*, Humana Press (2013); and in Cutler, *Protein Purification Protocols (Methods in Molecular Biology)*, Humana Press (2010).

5 In some embodiments, a gene modifying system, polypeptide, and/or template nucleic acid (e.g., template RNA) conforms to certain quality standards. In some embodiments, a gene modifying system, polypeptide, and/or template nucleic acid (e.g., template RNA) produced by a method described herein conforms to certain quality standards. Accordingly, the disclosure is directed, in some aspects, to methods of manufacturing a gene modifying system, polypeptide,
10 and/or template nucleic acid (e.g., template RNA) that conforms to certain quality standards, e.g., in which said quality standards are assayed. The disclosure is also directed, in some aspects, to methods of assaying said quality standards in a gene modifying system, polypeptide, and/or template nucleic acid (e.g., template RNA). In some embodiments, quality standards include, but are not limited to, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12) of the following:

15 (i) the length of the template RNA, e.g., whether the template RNA has a length that is above a reference length or within a reference length range, e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the template RNA present is greater than 100, 125, 150, 175, or 200 nucleotides long;

(ii) the presence, absence, and/or length of a polyA tail on the template RNA, e.g.,
20 whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the template RNA present contains a polyA tail (e.g., a polyA tail that is at least 5, 10, 20, 30, 50, 70, 100 nucleotides in length);

(iii) the presence, absence, and/or type of a 5' cap on the template RNA, e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the template RNA present contains a 5' cap, e.g., whether that cap is a 7-methylguanosine cap, e.g., a O-Me-m7G cap;
25

(iv) the presence, absence, and/or type of one or more modified nucleotides (e.g., selected from pseudouridine, dihydrouridine, inosine, 7-methylguanosine, 1-N-methylpseudouridine (1-Me-Ψ), 5-methoxyuridine (5-MO-U), 5-methylcytidine (5mC), or a locked nucleotide) in the template RNA, e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the
30 template RNA present contains one or more modified nucleotides;

- (v) the stability of the template RNA (e.g., over time and/or under a pre-selected condition), e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the template RNA remains intact (e.g., greater than 100, 125, 150, 175, or 200 nucleotides long) after a stability test;
- 5 (vi) the potency of the template RNA in a system for modifying DNA, e.g., whether at least 1% of target sites are modified after a system comprising the template RNA is assayed for potency;
- (vii) the length of the polypeptide, first polypeptide, or second polypeptide, e.g., whether the polypeptide, first polypeptide, or second polypeptide has a length that is above a reference
10 length or within a reference length range, e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the polypeptide, first polypeptide, or second polypeptide present is greater than 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids long (and optionally, no larger than 2500, 2000, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, or 600 amino acids long);
- 15 (viii) the presence, absence, and/or type of post-translational modification on the polypeptide, first polypeptide, or second polypeptide, e.g., whether at least 80, 85, 90, 95, 96, 97, 98, or 99% of the polypeptide, first polypeptide, or second polypeptide contains phosphorylation, methylation, acetylation, myristoylation, palmitoylation, isoprenylation, glypyatyon, or lipoylation, or any combination thereof;
- 20 (ix) the presence, absence, and/or type of one or more artificial, synthetic, or non-canonical amino acids (e.g., selected from ornithine, β -alanine, GABA, δ -Aminolevulinic acid, PABA, a D-amino acid (e.g., D-alanine or D-glutamate), aminoisobutyric acid, dehydroalanine, cystathionine, lanthionine, Djenkolic acid, Diaminopimelic acid, Homoalanine, Norvaline, Norleucine, Homonorleucine, homoserine, O-methyl-homoserine and O-ethyl-homoserine,
25 ethionine, selenocysteine, selenohomocysteine, selenomethionine, selenoethionine, tellurocysteine, or telluromethionine) in the polypeptide, first polypeptide, or second polypeptide, e.g., whether at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the polypeptide, first polypeptide, or second polypeptide present contains one or more artificial, synthetic, or non-canonical amino acids;
- 30 (x) the stability of the polypeptide, first polypeptide, or second polypeptide (e.g., over time and/or under a pre-selected condition), e.g., whether at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, or 99% of the polypeptide, first polypeptide, or second polypeptide remains intact (e.g., greater than 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1600, 1700, 1800, 1900, or 2000 amino acids long (and optionally, no larger than 2500, 2000, 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, or 600 amino acids long)) after a stability test;

(xi) the potency of the polypeptide, first polypeptide, or second polypeptide in a system for modifying DNA, e.g., whether at least 1% of target sites are modified after a system comprising the polypeptide, first polypeptide, or second polypeptide is assayed for potency; or

(xii) the presence, absence, and/or level of one or more of a pyrogen, virus, fungus, bacterial pathogen, or host cell protein, e.g., whether the system is free or substantially free of pyrogen, virus, fungus, bacterial pathogen, or host cell protein contamination.

In some embodiments, a system or pharmaceutical composition described herein is endotoxin free.

In some embodiments, the presence, absence, and/or level of one or more of a pyrogen, virus, fungus, bacterial pathogen, and/or host cell protein is determined. In embodiments, whether the system is free or substantially free of pyrogen, virus, fungus, bacterial pathogen, and/or host cell protein contamination is determined.

In some embodiments, a pharmaceutical composition or system as described herein has one or more (e.g., 1, 2, 3, or 4) of the following characteristics:

(a) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) DNA template relative to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;

(b) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) uncapped RNA relative to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;

(c) less than 1% (e.g., less than 0.5%, 0.4%, 0.3%, 0.2%, or 0.1%) partial length RNAs relative to the template RNA and/or the RNA encoding the polypeptide, e.g., on a molar basis;

(d) substantially lacks unreacted cap dinucleotides.

EXAMPLES

The invention is further illustrated by the following examples. The examples are provided for illustrative purposes only and are not to be construed as limiting the scope or content of the invention in any way.

Example 1: Gene modifying systems comprising Cas-Pol fusions with various Pol domains to enable precise editing in human cells

This example describes the ability of Cas-Pol fusions to programmably install mutations in genomic DNA in human cells. More specifically, the polymerase domain of Cas-Pol fusions, e.g., a polymerase domain described in this application, e.g., the human DNA Pol θ , is varied to determine the genome editing capacity of Cas-Pol fusions employing novel polymerase combinations. Template nucleic acids are co-delivered with Cas-Pol expression plasmids in human cells to determine the target editing activity of Cas-Pol fusions.

In order to generate domain libraries for genome engineering polypeptides, Cas effector proteins are selected; see in Table 3 and Table 4. Additional Cas9 domains are further selected for use in the genome engineering polypeptides described herein, as features including PAM requirements of a target sequence, predicted mutations for conferring nickase activity (e.g., D10A, H840A, or N863A for SpCas9), and gRNA features including single-guide composition, e.g., specific spacer parameters and gRNA scaffold sequence for conferring polypeptide binding for the cognate Cas enzyme, are able to be determined (Table 8). Linker sequences to connect Cas and Pol domains are collected based on a search for diversity of length, flexibility, and composition in order to optimize fusion proteins (Table 6). Polymerase domains are mined from a variety of sources using literature and polymerase protein domain signatures as described in this application, including wild-type polymerases capable of RNA and/or DNA-dependent DNA polymerization activity, derived polymerases with improved properties (e.g., thermostability, processivity, fidelity), derived polymerases with inactivated or reactivated functional domains (e.g., inactivated or reactivated domains conferring 5'-3' or 3'-5' exonuclease activity, proofreading activity, helicase activity, or RNase activity), and polymerases with synthetically evolved RNA-dependent and/or DNA-dependent DNA polymerase activity (e.g., the RTX polymerase derived from PolB of *Thermococcus kodakarensis* (KOD), as described in Ellefson et al Science 352(6293):1590-1593 (2016), incorporated herein by reference in its entirety).

Specifically, to assess the use of novel Pol domains in the context of a gene modifying polypeptide to successfully edit the genome, a subset of exemplary Pol domains are selected for fusion to a Cas9(N863A) nickase. Briefly, the protein sequences of monomeric human

polymerases are determined using UniProt (The UniProt Consortium Nucleic Acids Res 49(D1):D480-D489 (2021)) and functional domains further predicted and annotated using InterPro (Blum et al Nucleic Acids Res 49(D1):D344-D354 (2021)) and InterProScan (Jones et al Bioinformatics 30(9):1236-1240 (2014)) (Table X therein). Though not wishing to be limited
 5 by such example, proteins from the polymerase families Y, X, and A have been described that comprise a single subunit (see, e.g., Hoitsma et al Cell Mol Life Sci 77(1):35-59 (2020), incorporated herein by reference in its entirety).

To generate precise edits using genome engineering system Cas-Pol fusions, Template nucleic acids are constructed to template polymerization of an edit into the genomic target site by
 10 the Pol domain. Template nucleic acids are designed to comprise (i) a gRNA spacer sequence for guiding the Cas-Pol to the target region, e.g., a sequence complementary to a 20-nucleotide sequence in the HEK3 locus; (ii) a primer-binding sequence (PBS) capable of complementary base pairing with a single strand of the nicked DNA for target-primed polymerization; (iii) a
 15 heterologous object sequence providing a template for polymerization that further comprises the intended final target sequence; and (iv) a gRNA scaffold sequence to associate with the Cas9 domain of the Cas9-Pol polypeptide fusion. The constructs employed here specifically follow the 5' to 3' orientation (i), (iv), (iii), (ii). In some embodiments, (iii) may comprise RNA and/or DNA nucleotides. In some embodiments, (ii) may comprise RNA and/or DNA nucleotides. Without
 20 wishing to be limited by example, (i) and (iv) comprise RNA nucleotides in these experiments. Template compositions are described in Table 21 (Templates P1, P2, P3), where (ii) and (iii) may each be included as either RNA or DNA nucleotides. Template molecules optionally further comprise a 5' cap and 3' polyA tail.

Table 20: Template nucleic acids and second-nick gRNA used in Example 1

Name	Description	Spacer (i)	Scaffold (iv)	Pol template (iii)	PBS (ii)	Full Template Molecule
Template P1	HEK3_8PBS_10R T(CTTat1)	GGCCCAGACTGAGCAGC TGA	GTTT TAGAGCTA GAAATAGCAAGT TAAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCGAGTC GGTGC	TCTGCCATCAAAG	CGTGCT CA	GGCCCAGACTGAGCAGCTGA GTTT TAGAGCTAGAAATAGCA AGTTAAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTCTGCCAT CAAAGCGTGCTCA

Name	Description	Spacer (i)	Scaffold (iv)	Pol template (iii)	PBS (ii)	Full Template Molecule
Template P2	HEK3_13PBS_10 RT(CTTat1)	GGCCCAGACTGAGCAGC TGA	GTTTATAGAGCTA GAAATAGCAAGT TAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCAGAGTC GGTGC	TCTGCCATCAAAG	CGTGCT CAGTCT G	GGCCCAGACTGAGCAGCTGA GTTTATAGAGCTAGAAATAGCA AGTTAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTCTGCCAT CAAAGCGTGCTCAGTCTG
Template P3	HEK3_17PBS_10 RT(CTTat1)	GGCCCAGACTGAGCAGC TGA	GTTTATAGAGCTA GAAATAGCAAGT TAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCAGAGTC GGTGC	TCTGCCATCAAAG	CGTGCT CAGTCT GGGCC	GGCCCAGACTGAGCAGCTGA GTTTATAGAGCTAGAAATAGCA AGTTAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTCTGCCAT CAAAGCGTGCTCAGTCTGGG CC
Template P4	HBB_13PBS_10R T(TtoAat4)	GCATGGTGCACCTGACTC CTG	GTTTATAGAGCTA GAAATAGCAAGT TAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCAGAGTC GGTGC	AGACTTCTCCACAG	GAGTCA GGTGCA C	GCATGGTGCACCTGACTCCTG GTTTATAGAGCTAGAAATAGCA AGTTAAATAAGGCTAGTCCG TTATCAACTTGAAAAAGTGGC ACCGAGTCGGTGCTCTGCCAT CCACAGGAGTCAGGTGCAC
2gRNA P5	HEK3_+90	GTCAACCAGTATCCCGGT GC	GTTTATAGAGCTA GAAATAGCAAGT TAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCAGAGTC GGTGC	NA	NA	NA
2gRNA P6	HBB_+72	GCCTTGATACCAACCTGC CCA	GTTTATAGAGCTA GAAATAGCAAGT TAAATAAGGCT AGTCCGTTATCA ACTTGAAAAAGT GGCACCAGAGTC GGTGC	NA	NA	NA

U2OS or HEK293T cells are transfected by electroporation of 250,000 cells/well with ~800 ng of Cas9-Pol fusion (e.g., pol theta fusion) expression plasmid, 200 ng of a chemically synthesized template nucleic acid molecule, and optionally 83 ng of an additional second-nick gRNA (2gRNA P5 for Templates P1, P2, P3 or 2gRNA P6 for Template P4) (Table 21). To assess the genome editing capacity of Cas-Pol fusions, genomic DNA (gDNA) is collected on day 3 post-transfection. The frequencies of intended (exact and scarless edit as designed) and unintended (any non-intended changes to the target sequence) edits at target loci (HEK3 for Templates P1, P2, P3 or HBB for Template P4) are analyzed by amplicon sequencing. As used herein, amplicon sequencing of a target site comprises the use of site-specific primers in PCR amplification of the target site, sequencing of amplicons on an Illumina MiSeq, and detection and characterization of editing events using the CRISPResso2 pipeline (Clement et al Nat Biotechnol 37(3):224-226 (2019)). In some embodiments, active Cas-Pol fusions result in

detectable levels of edits, e.g., at least 0.1% of sequencing reads demonstrate the target site edit. In some embodiments, desirable Cas-Pol fusions demonstrate a higher frequency of intended to unintended edits, e.g., at least 2-fold higher frequency of intended edits to unintended edits.

Example 2: Improvement of expression of Cas-Pol fusions through linker selection

5 This example illustrates the optimization of Cas-Pol fusions to improve protein expression in mammalian cells. Construction of novel Cas-Pol fusions by the substitution of new functional domains as described in Example 1 above may result in low or moderate expression of the genome engineering polypeptide. Thus, it is contemplated here that modified configurations of the fusions may be advantageous in the context of different domains. Without
10 wishing to be limited by the example, one such approach for improving the expression and stability of new fusions is through the use of a linker library. Here, the peptide linker sequence between the Cas and Pol domains of a Cas-Pol fusion is varied using a library of linker sequences. More specifically, linkers from Table 21 below are used to generate new variants of a Cas9 fusion constructs and delivered to human cells to screen for improved Cas-Pol protein
15 expression.

A set of 22 peptide linkers (Table 21) with varying degrees of length, flexibility, hydrophobicity, and secondary structure are first used to generate variants of a Cas-Pol fusion protein by substitution of the original linker (see example 30 referenced above). HEK293T cells are transfected by electroporation of 250,000 cells/well with ~800 ng of each Cas9-Pol fusion
20 plasmid along with 200 ng of a single-guide RNA plasmid. To assess the expression level of Cas9-Pol fusions, cell lysates are collected on day 2 post-transfection and analyzed by Western blot using a primary antibody against Cas9.

Table 21. Peptide sequences for use as linkers between the Cas and Pol domains in genome engineering polypeptides comprising Cas-Pol fusions

#	Linker sequence	Notes
1	GGS	Short
2	GGGGS	Flexible, short
3	(GGGGS)2	Flexible
4	(GGGGS)3	Flexible, long
5	(GGGGS)4	Flexible, very long
6	(G)6	Flexible
7	(G)8	Flexible
8	GSAGSAAGSGEF	Flexible
9	(GSSGSS)	Mid
10	(GSSGSS)2	Mid, Flexible
11	(GSSGSS)3	Mid

12	SGSETPGTSESATPES	XTEN
13	(EAAAK)	Rigid helix, short
14	(EAAAK)2	Rigid helix, mid
15	(EAAAK)3	Rigid helix, long
16	PAP	Rigid, short
17	PAPAP	Rigid, short
18	PAPAPAPAP	Rigid, mid
19	A(EAAAK)4ALEA(EAAAK)4A	Rigid, very long with helices
20	GGGGS(EAAAK)GGGGS	Flexible – helix – flex
21	(EAAAK)GGGGS(EAAAK)	Helix – flex – helix
22	SGGSSGGSSSGSETPGTSESATPESSGGSSGGSS	Flexible – XTEN – flexible

Example 3: Gene modifying polypeptide selection by pooled screening in HEK293T & U2OS cells

This example describes the use of an RNA gene modifying system for the targeted editing of a coding sequence in the human genome. More specifically, this example describes the infection of HEK293T and U2OS cells with a library of gene modifying candidates, followed by transfection of a template guide RNA (tgRNA) for *in vitro* gene modifying in the cells, e.g., as a means of evaluating a new gene modifying polypeptide for editing activity in human cells by a pooled screening approach.

The gene modifying polypeptide library candidates assayed herein each comprise: 1) a *S. pyogenes* (Spy) Cas9 nickase containing an N863A mutation that inactivates one endonuclease active site; 2) one of the 122 peptide linkers depicted at **Table 6**; and 3) a reverse transcriptase (RT) domain from **Table 2** of retroviral origin. The particular retroviral RT domains utilized were selected if they were expected to function as a monomer. For each selected RT domain, the wild-type sequences were tested, as well as versions with point mutations installed in the primary wild-type sequence. In particular, 143 RT domains were tested, either wild type or containing various mutations. In total, 17,446 Cas-linker-RT gene modifying polypeptides were tested.

The system described here is a two-component system comprising: 1) an expression plasmid encoding a human codon-optimized gene modifying polypeptide library candidate within a lentiviral cassette, and 2) a tgRNA expression plasmid expressing a non-coding tgRNA sequence that is recognized by Cas and localizes it to the genomic locus of interest, and that also templates reverse transcription of the desired edit into the genome by the RT domain, driven by a U6 promoter. The lentiviral cassette comprises: (i) a CMV promoter for expression in mammalian cells; (ii) a gene modifying polypeptide library candidate as shown; (iii) a self-cleaving T2A

polypeptide; (iv) a puromycin resistance gene enabling selection in mammalian cells; and (v) a polyA tail termination signal.

To prepare a pool of cells expressing gene modifying polypeptide library candidates, HEK293T or U2OS cells were transduced with pooled lentiviral preparations of the gene
 5 modifying candidate plasmid library. HEK293 Lenti-X cells were seeded in 15 cm plates (12×10^6 cells) prior to lentiviral plasmid transfection. Lentiviral plasmid transfection using the Lentiviral Packaging Mix (Biosettia, 27 ug) and the plasmid DNA for the gene modifying candidate library (27 ug) was performed the following day using Lipofectamine 2000 and Opti-MEM media according to the manufacturer's protocol. Extracellular DNA was removed by a full media change
 10 the next day and virus-containing media was harvested 48 hours after. Lentiviral media was concentrated using Lenti-X Concentrator (TaKaRa Biosciences) and 5 mL lentiviral aliquots were made and stored at -80°C . Lentiviral titering was performed by enumerating colony forming units post Puromycin selection. HEK293T or U2OS cells carrying a BFP-expressing genomic landing pad were seeded at 6×10^7 cells in culture plates and transduced at a 0.3 multiplicity of infection
 15 (MOI) to minimize multiple infections per cell. Puromycin (2.5 ug/mL) was added 48 hours post infection to allow for selection of infected cells. Cells were kept under puromycin selection for at least 7 days and then scaled up for tgRNA electroporation.

To determine the genome-editing capacity of the gene modifying library candidates in the assay, infected BFP-expressing HEK293T or U2OS cells were then transfected by electroporation
 20 of 250,000 cells/well with 200 ng of a tgRNA (either g4 or g10) plasmid, designed to convert BFP to GFP, at sufficient cell count for $>1000\times$ coverage per library candidate.

The g4 tgRNA (5' to 3') is as follows: 20 nucleotide spacer region (GCCGAAGCACTGCACGCCGT), a scaffold region (GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAA
 25 AAGTGGCACCGAGTCGGTGC), the template region encoding the single base pair substitution to change BFP to GFP (**bold**) and a PAM inactivation that introduces a synonymous point mutation in the SpyCas9 PAM (NGG to NCG) that prevents re-engagement of the gene modifying polypeptide upon completion of a functional gene modifying reaction (underline) (ACCCTGACTACG), and the 13 nucleotide PBS (GCGTGCAGTGCTT).

30 Similarly, the g10 tgRNA (5' to 3') is as follows: 20 nucleotide spacer region (AGAAGTCGTGCTGCTTCATG), a scaffold region

(GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAA
 AAGTGGCACCAGTCGGTGC), the template region encoding the single base pair
 substitution to change BFP to GFP (**bold**) and a PAM inactivation that introduces a synonymous
 point mutation in the SpyCas9 PAM (NGG to NGA) that prevents re-engagement of the gene
 5 modifying polypeptide upon completion of a functional gene modifying reaction (underline)
 (ACCCTGACCTACGGCGTGCAGTGCTTCGGCCGCTACCCCGATCACAT), and 13
 nucleotide PBS (GAAGCAGCACGAC).

To assess the genome-editing capacity of the various constructs in the assay, cells were
 sorted by Fluorescence-Activated Cell Sorting (FACS) for GFP expression 6-7 days post-
 10 electroporation. Cells were sorted and harvested as distinct populations of unedited (BFP+) cells,
 edited (GFP+) cells and imperfect edit (BFP-, GFP-) cells. A sample of unsorted cells was also
 harvested as the input population to determine enrichment during analysis.

To determine which gene modifying library candidates have genome-editing capacity in
 this assay, genomic DNA (gDNA) was harvested from sorted and unsorted cell populations, and
 15 analyzed by sequencing the gene modifying library candidates in each population. Briefly, gene
 modifying sequences were amplified from the genome using primers specific to the lentiviral
 cassette, amplified in a second round of PCR to dilute genomic DNA, and then sequenced using
 Oxford Nanopore Sequencing Technology according to the manufacturer's protocol.

After quality control of sequencing reads, reads of at least 1500 and no more than 3200
 20 nucleotides were mapped to the gene modifying polypeptide library sequences and those
 containing a minimum of an 80% match to a library sequence were considered to be successfully
 aligned to a given candidate. To identify gene modifying candidates capable of performing gene
 editing in the assay, the read count of each library candidate in the edited population was compared
 to its read count in the initial, unsorted population. For purposes of this pooled screen, gene
 25 modifying candidates with genome-editing capacity were selected as those candidates that were
 enriched in the converted (GFP+) population relative to unsorted (input) cells and wherein the
 enrichment was determined to be at or above the enrichment level of a reference (Element ID No:
 17380).

A large number of gene modifying polypeptide candidates were determined to be enriched
 30 in the GFP+ cell populations. For example, of the 17,446 candidates tested, over 3,300 exhibited
 enrichment in GFP+ sorted populations (relative to unsorted) that was at least equivalent to that of

the reference under similar experimental conditions (HEK293T using g4 tgRNA; HEK293T cells using g10 tgRNA; or U2OS cells using g4 tgRNA), shown in Table D. Although the 17,446 candidates were also tested in U2OS cells using g10 tgRNA, the pooled screen did not yield candidates that were enriched in the converted (GFP+) population relative to unsorted (input) cells under that experimental condition; further investigation is required to explain these results.

Table D. Combinations of linker and RT sequences screened. The amino acid sequence of each RT in this table is provided in Table 6.

Linker amino acid sequence	RT domain name
EAAAKGSS	PERV_Q4VFZ2_3mutA_WS
EAAAKEAAKEAAKEAAK	MLVMS_P03355_PLV919
PAPEAAAK	MLVFF_P26809_3mutA
EAAAKPAPGGG	MLVFF_P26809_3mutA
GSSGSSGSSGSSGSSGSS	PERV_Q4VFZ2_3mut
PAPGGGEAAK	MLVAV_P03356_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAK	MLVMS_P03355_PLV919
GSSEAAK	MLVFF_P26809_3mutA
EAAAKPAPGGS	MLVFF_P26809_3mutA
GGSGSGSGSGSGSGSGGS	MLVFF_P26809_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAK	XMRV6_A1Z651_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKEAAKEAAK	MLVFF_P26809_3mutA
PAPEAAKGSS	MLVFF_P26809_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAK	AVIRE_P03360_3mutA
PAPAPAPAPAP	MLVCB_P08361_3mutA
PAPAPAPAPAP	MLVFF_P26809_3mutA
EAAAKGGSPAP	PERV_Q4VFZ2_3mutA_WS
PAP	MLVMS_P03355_PLV919
PAPGGGGSS	WMSV_P03359_3mutA
SGSETPGTSESATPES	MLVFF_P26809_3mutA
PAPEAAKGSS	XMRV6_A1Z651_3mutA
EAAAKGGSGGG	MLVMS_P03355_PLV919
GGGSGGGGS	MLVFF_P26809_3mutA
GGGPAPGSS	MLVAV_P03356_3mutA
GGSGSGSGSGSGSGSGGS	XMRV6_A1Z651_3mut
GGGSGSGSGSGSGSGSGSGSGSGSGGS	MLVCB_P08361_3mutA
GSSPAP	AVIRE_P03360_3mutA

Linker amino acid sequence	RT domain name
EAAAKGSSPAP	MLVFF_P26809_3mutA
GSSGGGEAAAK	MLVFF_P26809_3mutA
GGSGSGSGSGSGSGSGS	MLVMS_P03355_3mutA_WS
PAPAPAPAP	MLVFF_P26809_3mutA
EAAAKEAAKEAAKEAAK	XMRV6_A1Z651_3mutA
EAAAKGGSPAP	MLVMS_P03355_3mutA_WS
PAPGGSEAAAK	AVIRE_P03360_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	AVIRE_P03360_3mutA
EAAAKGGGSEAAAK	MLVCB_P08361_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	WMSV_P03359_3mutA
GSS	MLVMS_P03355_PLV919
GSSGSSGSSGSS	MLVMS_P03355_PLV919
GSSPAPEAAAK	XMRV6_A1Z651_3mutA
GGSPAPEAAAK	MLVFF_P26809_3mutA
GGGEAAAKGGS	MLVFF_P26809_3mutA
EAAAKEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
GGGGGGGG	PERV_Q4VFZ2_3mut
GGGPAP	MLVCB_P08361_3mutA
PAPAPAPAPAPAP	MLVCB_P08361_3mutA
GGSGSGSGSGSGSGSGS	MLVCB_P08361_3mutA
PAP	MLVMS_P03355_3mutA_WS
GGSGSGSGSGSGSGSGS	PERV_Q4VFZ2_3mutA_WS
PAPAPAPAPAPAP	MLVMS_P03355_PLV919
EAAAKPAPGSS	MLVMS_P03355_3mutA_WS
EAAAKEAAKEAAKEAAK	MLVMS_P03355_3mutA_WS
EAAAKGGS	MLVMS_P03355_3mutA_WS
GGGGSEAAAKGGGGS	MLVFF_P26809_3mutA
EAAAKPAPGSS	MLVFF_P26809_3mutA
GGGGSGGGSGGGSGGGGS	MLVMS_P03355_PLV919
EAAAKGGGGS	MLVMS_P03355_PLV919
GGSPAP	XMRV6_A1Z651_3mutA
EAAAKGGGPAP	MLVMS_P03355_PLV919
EAAAKEAAKEAAKEAAKEAAK	MLVFF_P26809_3mutA
PAP	MLVCB_P08361_3mutA
EAAAK	XMRV6_A1Z651_3mutA
GGSGSSPAP	PERV_Q4VFZ2_3mutA_WS
GSSGSSGSSGSSGSSGSS	MLVMS_P03355_PLV919
GSSEAAAKGGG	MLVAV_P03356_3mutA
GGGEAAAKGGS	XMRV6_A1Z651_3mutA
EAAAKGGGSEAAAK	MLVAV_P03356_3mutA

Linker amino acid sequence	RT domain name
GGGGSGGGSGGGGS	MLVFF_P26809_3mutA
GGGGSGGGSGGGSGGGGS	AVIRE_P03360_3mutA
SGSETPGTSESATPES	AVIRE_P03360_3mutA
GGGEAAAKPAP	MLVFF_P26809_3mutA
EAAAKGSSGGG	MLVMS_P03355_3mutA_WS
EAAAKEAAKEAAKEAAKEAAK	WMSV_P03359_3mut
GGSGSGSGSGGS	XMRV6_A1Z651_3mutA
GGSEAAAKPAP	MLVFF_P26809_3mutA
EAAAKGSSGGG	XMRV6_A1Z651_3mutA
GGGGS	MLVFF_P26809_3mutA
GGGEAAAKGSS	MLVMS_P03355_PLV919
PAPAPAPAPAPAP	MLVAV_P03356_3mutA
GGGGSGGGSGGGSGGGGS	MLVCB_P08361_3mutA
GGGEAAAKGSS	MLVCB_P08361_3mutA
PAPGGSGSS	MLVFF_P26809_3mutA
GSAGSAAGSGEF	MLVCB_P08361_3mutA
PAPGGSEAAAK	MLVMS_P03355_3mutA_WS
GGSGSS	XMRV6_A1Z651_3mutA
PAPGGGGSS	MLVMS_P03355_PLV919
GSSGSSGSS	XMRV6_A1Z651_3mut
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MLVMS_P03355_3mutA_WS
EAAAK	MLVMS_P03355_PLV919
GSSGSSGSSGSS	MLVFF_P26809_3mutA
PAPGGGGSS	MLVCB_P08361_3mutA
GGGEAAAKGSS	MLVCB_P08361_3mutA
PAPGGGEAAAK	MLVMS_P03355_PLV919
GGGGGSPAP	XMRV6_A1Z651_3mutA
EAAAKGSS	XMRV6_A1Z651_3mutA
EAAAKGSSPAP	XMRV6_A1Z651_3mut
PAPEAAK	MLVAV_P03356_3mutA
GGSGSGSGSGGS	MLVMS_P03355_3mutA_WS
GGGPAPGSS	MLVMS_P03355_PLV919
GSSGSSGSSGSS	PERV_Q4VFZ2_3mutA_WS
EAAAKPAPGSS	MLVCB_P08361_3mutA
GSSGSS	MLVFF_P26809_3mutA
EAAAKEAAKEAAKEAAK	MLVCB_P08361_3mutA
EAAKEAAKEAAKEAAK	FLV_P10273_3mutA
GSS	MLVFF_P26809_3mutA
EAAAKEAAK	MLVMS_P03355_3mutA_WS
PAPEAAKGGG	MLVAV_P03356_3mutA

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Linker amino acid sequence	RT domain name
GGGGGGG	MLVCB_P08361_3mutA
GSSGSSGSSGSSGSSGSS	MLVCB_P08361_3mutA
GGGPAPGGS	MLVFF_P26809_3mutA
EAAAKGGSGGG	PERV_Q4VFZ2_3mut
EAAAKGGGGSS	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSSGSSGSS	MLVMS_P03355_3mut
GGGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mutA_WS
PAPAPAPAPAP	MLVMS_P03355_PLV919
GGGEAAAKGGS	MLVMS_P03355_PLV919
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MLVMS_P03355_3mut
GSAGSAAGSGEF	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSSGSS	MLVFF_P26809_3mutA
EAAAKGGSGSS	MLVFF_P26809_3mutA
PAPGGG	MLVFF_P26809_3mutA
GGGPAPGSS	XMRV6_A1Z651_3mutA
PAPEAAAKGGS	AVIRE_P03360_3mutA
PAPGGGEAAAK	MLVFF_P26809_3mut
GGGGSSEAAAK	MLVCB_P08361_3mutA
EAAAK	MLVMS_P03355_PLV919
GGGGSGGGSGGGSGGGSGGGSGGGGS	BAEVM_P10272_3mutA
GGSGGGEAAAK	MLVMS_P03355_PLV919
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MLVFF_P26809_3mutA
GSSPAPGGS	XMRV6_A1Z651_3mutA
GGSGGGPAP	MLVMS_P03355_PLV919
EAAAK	AVIRE_P03360_3mutA
GSS	XMRV6_A1Z651_3mutA
GGSGSGSGS	MLVFF_P26809_3mutA
EAAKEAAKEAAKEAAK	AVIRE_P03360_3mut
PAPEAAAKGGG	PERV_Q4VFZ2_3mutA_WS
GGGGGSEAAAK	BAEVM_P10272_3mutA
GGSGSSGGG	MLVMS_P03355_3mutA_WS
GGGGGGG	MLVMS_P03355_3mutA_WS
GSSEAAAKPAP	PERV_Q4VFZ2_3mut
GGGGGSEAAAK	WMSV_P03359_3mut
GGGGSGGGSGGGSGGGSGGGGS	MLVFF_P26809_3mut
GGGEAAAKGGS	AVIRE_P03360_3mutA
GGSPAPGGG	AVIRE_P03360_3mutA
GSAGSAAGSGEF	MLVAV_P03356_3mutA
EAAAK	MLVAV_P03356_3mutA
EAAAKPAPGSS	WMSV_P03359_3mutA

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Linker amino acid sequence	RT domain name
PAPGGSEAAK	MLVCB_P08361_3mutA
PAPGSSEAAK	MLVBM_Q7SVK7_3mutA_WS
PAPEAAKGSS	AVIRE_P03360_3mutA
GGSPAPGSS	WMSV_P03359_3mutA
PAPGGSGGG	MLVMS_P03355_PLV919
EAAKGGSGSS	MLVMS_P03355_3mutA_WS
GGSGGG	MLVFF_P26809_3mutA
GGSEAAKGSS	KORV_Q9TTC1_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MLVCB_P08361_3mutA
PAPAPAPAPAPAP	PERV_Q4VFZ2_3mutA_WS
PAPEAAK	MLVMS_P03355_3mutA_WS
GGSEAAKGGG	MLVMS_P03355_PLV919
GSSPAP	MLVMS_P03355_3mutA_WS
GGGGSS	MLVMS_P03355_PLV919
GGGEAAKPAP	AVIRE_P03360_3mutA
EAAKPAPGSS	MLVAV_P03356_3mutA
EAAKGGGPAP	MLVAV_P03356_3mutA
PAPGGSEAAK	BAEVM_P10272_3mutA
PAPGGSGSS	MLVMS_P03355_3mutA_WS
PAPGGSGSS	AVIRE_P03360_3mutA
GGSGGGPAP	MLVMS_P03355_3mutA_WS
EAAKEAAKEAAKEAAK	BAEVM_P10272_3mutA
GGGGSGGGSGGGSGGGSGGGSS	MLVMS_P03355_PLV919
GGGGSSPAP	MLVCB_P08361_3mutA
GSSGGGPAP	MLVFF_P26809_3mutA
GGGGSSGSS	MLVMS_P03355_PLV919
GGSGGG	MLVCB_P08361_3mutA
GSSGGGGSS	MLVMS_P03355_PLV919
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	XMRV6_A1Z651_3mutA
GGGGSGSS	KORV_Q9TTC1_3mut
GGGEAAKGSS	BAEVM_P10272_3mutA
GGSGGG	BAEVM_P10272_3mutA
PAPAPAP	KORV_Q9TTC1-Pro_3mut
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	SFV3L_P27401_2mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MLVBM_Q7SVK7_3mutA_WS
GSSGSSGSSGSSGSS	MLVMS_P03355_3mutA_WS
GSSGGGEAAK	MLVMS_P03355_3mutA_WS
GSSGGSEAAK	MLVFF_P26809_3mutA
PAP	MLVMS_P03355_PLV919
EAAKGGGGSEAAK	MLVBM_Q7SVK7_3mutA_WS

Linker amino acid sequence	RT domain name
PAPAP	AVIRE_P03360_3mutA
PAP	MLVFF_P26809_3mutA
GSSGGG	MLVMS_P03355_3mut
GSSPAPGGS	MLVFF_P26809_3mutA
PAPAPAPAP	XMRV6_A1Z651_3mutA
EAAAKGSSGGS	PERV_Q4VFZ2_3mut
PAPEAAKGGG	KORV_Q9TTC1-Pro_3mutA
PAPGGS	MLVCB_P08361_3mutA
EAAAKGGG	MLVCB_P08361_3mutA
GSSEAAKPAP	MLVMS_P03355_PLV919
PAPGGS	MLVFF_P26809_3mutA
EAAAKGGS	MLVCB_P08361_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	FLV_P10273_3mutA
PAPGGSEAAK	MLVAV_P03356_3mutA
GSS	MLVCB_P08361_3mutA
GSSGSSGSSGSS	AVIRE_P03360_3mutA
GSSGSSGSS	MLVFF_P26809_3mutA
GSSGGG	MLVMS_P03355_PLV919
EAAAK	MLVFF_P26809_3mutA
GGSPAPEAAK	MLVCB_P08361_3mutA
GGSGSS	MLVCB_P08361_3mutA
GSSPAPGGG	MLVMS_P03355_PLV919
EAAKEAAKEAAKEAAKEAAK	MLVAV_P03356_3mutA
EAAAKGSSPAP	FLV_P10273_3mutA
GGGGSS	XMRV6_A1Z651_3mutA
GGSPAPGSS	MLVMS_P03355_PLV919
EAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_3mutA_WS
PAPEAAKGGG	FLV_P10273_3mutA
EAAAKPAPGGS	XMRV6_A1Z651_3mut
PAPAP	BAEVM_P10272_3mutA
EAAKEAAKEAAKEAAK	MLVMS_P03355_PLV919
GSSPAPGGG	MLVMS_P03355_PLV919
EAAKGGGPAP	KORV_Q9TTC1_3mutA
PAPEAAK	MLVMS_P03355_PLV919
PAPGGGEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKGSSGGS	MLVMS_P03355_3mutA_WS
EAAKEAAKEAAK	MLVMS_P03355_PLV919
GSSEAAK	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSS	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGSGGGGS	MLVMS_P03355_3mutA_WS

Linker amino acid sequence	RT domain name
EAAAKGGGSEAAK	MLVMS_P03355_3mut
GGS	MLVCB_P08361_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	XMRV6_A1Z651_3mutA
GGSGSSPAP	MLVCB_P08361_3mutA
GGGGSGGGSGGGGS	XMRV6_A1Z651_3mutA
PAPAPAPAPAP	BAEVM_P10272_3mutA
PAPAPAPAPAP	MLVMS_P03355_3mutA_WS
EAAKEAAKEAAKEAAK	MLVBM_Q7SVK7_3mut
GGGGSGGGSGGGSGGGSGGGGS	BAEVM_P10272_3mutA
GGSGSGGS	MLVMS_P03355_3mutA_WS
EAAAKPAPGSS	MLVMS_P03355_PLV919
GSS	MLVMS_P03355_3mutA_WS
PAPEAAKGGG	MLVMS_P03355_3mutA_WS
GGGPAPGGS	MLVMS_P03355_3mutA_WS
EAAAKGGGSS	MLVAV_P03356_3mutA
GSSGSSGSSGSSGSS	MLVFF_P26809_3mut
SGSETPGTSESATPES	PERV_Q4VFZ2_3mut
GGSEAAKGGG	MLVMS_P03355_3mut
GSSGSSGSSGSSGSSGSS	AVIRE_P03360_3mutA
PAPAPAPAPAPAP	AVIRE_P03360_3mut
GGSGGS	XMRV6_A1Z651_3mutA
PAPGSSEAAK	MLVCB_P08361_3mut
GGSPAPEAAK	PERV_Q4VFZ2_3mut
EAAAKGGGGGS	MLVCB_P08361_3mutA
GGSGSGSGSGGS	MLVMS_P03355_PLV919
GGGGSEAAK	MLVMS_P03355_PLV919
GSSEAAKGGG	MLVFF_P26809_3mutA
PAPGGS	MLVMS_P03355_3mutA_WS
EAAAKGGSGGG	MLVCB_P08361_3mutA
EAAAKGGG	PERV_Q4VFZ2_3mut
PAPGGS	XMRV6_A1Z651_3mutA
GSSPAPGGG	XMRV6_A1Z651_3mutA
PAPEAAKGGG	MLVMS_P03355_3mutA_WS
GSSEAAKGGG	PERV_Q4VFZ2_3mutA_WS
PAPGGSSEAAK	XMRV6_A1Z651_3mutA
GGGGGS	MLVMS_P03355_3mutA_WS
GGSPAPEAAK	MLVMS_P03355_3mutA_WS
GGGPAP	MLVFF_P26809_3mutA
PAPGSSGGG	XMRV6_A1Z651_3mutA
PAPGSSGGG	MLVBM_Q7SVK7_3mutA_WS

Linker amino acid sequence	RT domain name
GGGEAAKGSS	MLVMS_P03355_3mutA_WS
GSSEAAKGSS	MLVCB_P08361_3mutA
PAPGGSGSS	MLVCB_P08361_3mutA
EAAAKGGGSEAAK	BAEVM_P10272_3mutA
PAPAPAP	PERV_Q4VFZ2_3mutA_WS
GGGGGG	MLVAV_P03356_3mutA
GSSPAPEAAK	MLVCB_P08361_3mutA
GGSGSGSGS	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSSGSS	XMRV6_A1Z651_3mut
GGGPAPGSS	XMRV6_A1Z651_3mutA
GGGPAPEAAK	BAEVM_P10272_3mutA
GGSGGG	AVIRE_P03360_3mutA
SGSETPGTSESATPES	PERV_Q4VFZ2_3mutA_WS
EAAAKGSSPAP	MLVMS_P03355_PLV919
GSSEAAK	XMRV6_A1Z651_3mut
GSSGSGGG	MLVFF_P26809_3mutA
EAAAKEAAKEAAKEAAKEAAK	WMSV_P03359_3mutA
GGGGSEAAKGGGGS	MLVMS_P03355_PLV919
PAPGGGGSS	MLVMS_P03355_3mutA_WS
SGSETPGTSESATPES	MLVMS_P03355_3mutA_WS
GGSPAPEAAK	KORV_Q9TTC1-Pro_3mutA
GSSEAAKGGG	MLVMS_P03355_3mutA_WS
GSSEAAK	WMSV_P03359_3mutA
GGGGSEAAKGGGGS	AVIRE_P03360_3mutA
GSS	WMSV_P03359_3mutA
PAPGGSEAAK	MLVFF_P26809_3mutA
GGGGS	MLVMS_P03355_3mutA_WS
GGGPAP	MLVMS_P03355_3mutA_WS
EAAAKEAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_3mutA_WS
EAAKPAPGSS	PERV_Q4VFZ2_3mut
EAAKPAPGSS	MLVCB_P08361_3mutA
GGGGG	WMSV_P03359_3mutA
EAAKPAPGGS	MLVMS_P03355_PLV919
PAPGGGEAAK	PERV_Q4VFZ2_3mut
EAAKEAAKEAAKEAAKEAAK	AVIRE_P03360_3mutA
GSSEAAKPAP	XMRV6_A1Z651_3mutA
PAPGGSEAAK	MLVBM_Q7SVK7_3mutA_WS
PAPGSS	MLVCB_P08361_3mutA
EAAKGGG	MLVMS_P03355_3mutA_WS
EAAKPAP	MLVCB_P08361_3mutA

Linker amino acid sequence	RT domain name
PAPEAAKGGG	MLVBM_Q7SVK7_3mutA_WS
GGSPAPGGG	MLVCB_P08361_3mutA
PAPGGSGSS	WMSV_P03359_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_PLV919
GGSGGGPAP	MLVMS_P03355_PLV919
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	MLVMS_P03355
PAPEAAKGGG	MLVCB_P08361_3mutA
EAAKGGG	MLVMS_P03355_3mutA_WS
GGSGGS	MLVMS_P03355_3mutA_WS
EAAKEAAKEAAKEAAKEAAK	BAEVM_P10272_3mutA
GGGGSEAAKGGGG	FLV_P10273_3mutA
GGSEAAKGGG	MLVCB_P08361_3mutA
GSSGSSGSSGSSGSS	BAEVM_P10272_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	MLVFF_P26809_3mutA
EAAKGGG	PERV_Q4VFZ2_3mut
GGGGSEAAK	MLVCB_P08361_3mutA
EAAKPAPGGG	MLVMS_P03355_3mutA_WS
GGGGGSGSS	XMRV6_A1Z651_3mutA
PAPGSSEAAK	MLVMS_P03355_3mutA_WS
GSSEAAKPAP	MLVCB_P08361_3mutA
EAAKGGSPAP	MLVAV_P03356_3mutA
GGGPAPGGG	WMSV_P03359_3mutA
GGSPAP	MLVMS_P03355_3mutA_WS
GGSEAAKGGG	MLVMS_P03355_3mutA_WS
GGGGGGG	MLVFF_P26809_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGSGGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mutA_WS
GSSPAPGGG	MLVAV_P03356_3mutA
GGGGGG	AVIRE_P03360_3mutA
GSSGGS	MLVMS_P03355_3mutA_WS
GGSPAPGSS	MLVFF_P26809_3mutA
PAPEAAKGGG	PERV_Q4VFZ2_3mut
EAAKGGGPAP	MLVFF_P26809_3mutA
GGGEAAKGGG	MLVMS_P03355_PLV919
GGSGSSPAP	MLVFF_P26809_3mutA
SGSETPGTSESATPES	WMSV_P03359_3mutA
PAPGGSEAAK	MLVBM_Q7SVK7_3mutA_WS
GGSGGG	MLVMS_P03355_PLV919
GGGGSSPAP	PERV_Q4VFZ2_3mut
GGGEAAKGGG	MLVAV_P03356_3mutA

Linker amino acid sequence	RT domain name
PAPAPAPAPAPAP	MLVMS_P03355_3mutA_WS
EAAAKGGGSEAAK	PERV_Q4VFZ2
EAAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_PLV919
GGGGSEAAK	PERV_Q4VFZ2_3mut
PAPGSSEAAK	MLVCB_P08361_3mutA
GSAGSAAGSGEF	PERV_Q4VFZ2_3mutA_WS
EAAAKGGGSEAAK	MLVFF_P26809_3mutA
GGSPAPGGG	PERV_Q4VFZ2_3mutA_WS
GSSEAAKGGG	AVIRE_P03360_3mutA
GGGEAAKPAP	MLVMS_P03355_3mutA_WS
GGGPAP	AVIRE_P03360_3mutA
GGSEAAK	MLVCB_P08361_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	PERV_Q4VFZ2_3mut
EAAKPAPGGS	MLVBM_Q7SVK7_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	XMRV6_A1Z651_3mut
GGGGGGGG	MLVCB_P08361_3mutA
PAPGSS	PERV_Q4VFZ2_3mut
EAAK	PERV_Q4VFZ2_3mut
GSAGSAAGSGEF	MLVMS_P03355_3mutA_WS
PAPGGGEAAK	PERV_Q4VFZ2_3mut
EAAKGSSGGS	MLVFF_P26809_3mut
GGGGSEAAKGGGGS	BAEVM_P10272_3mutA
GGGGSGGGSGGGGS	MLVMS_P03355_PLV919
EAAKGGGGSEAAK	BAEVM_P10272_3mut
PAPGGGEAAK	MLVMS_P03355_3mutA_WS
GGSEAAKPAP	MLVMS_P03355_3mutA_WS
PAPAP	MLVCB_P08361_3mutA
PAPAP	MLVFF_P26809_3mutA
GGSPAP	AVIRE_P03360_3mutA
EAAKGSSGGS	MLVCB_P08361_3mutA
PAPGSSGGS	AVIRE_P03360_3mutA
EAAKGGGGSEAAK	XMRV6_A1Z651_3mutA
PAPAPAP	BAEVM_P10272_3mutA
GGSGSGSGSGSGSGSGS	MLVMS_P03355_PLV919
GGGGSGSGS	MLVMS_P03355_PLV919
PAPGSSEAAK	XMRV6_A1Z651_3mut
GGSEAAKPAP	XMRV6_A1Z651_3mutA
EAAKEAAKEAAKEAAK	XMRV6_A1Z651_3mut
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	WMSV_P03359_3mut
GGGGGEAAK	XMRV6_A1Z651_3mutA

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Linker amino acid sequence	RT domain name
PAPGSSGGS	BAEVM_P10272_3mutA
GGGGGG	MLVFF_P26809_3mutA
PAPGGSEAAK	MLVMS_P03355_PLV919
PAPGGS	MLVMS_P03355_PLV919
GGSGSGSGSGGS	BAEVM_P10272_3mutA
GSSPAP	MLVCB_P08361_3mutA
PAPAPAPAP	MLVMS_P03355_3mutA_WS
GGGGGG	MLVCB_P08361_3mutA
GSSGSSGSSGSSGSSGSS	KORV_Q9TTC1-Pro_3mutA
GSSEAAKGGG	BAEVM_P10272_3mutA
GGSEAAK	FLV_P10273_3mutA
GGSGSGSGSGSGSGGS	KORV_Q9TTC1-Pro_3mutA
GSSPAPEAAK	PERV_Q4VFZ2_3mut
GSSGSSGSSGSSGSS	XMRV6_A1Z651_3mutA
EAAKPAPGGS	MLVMS_P03355_3mut
SGGSGGSSGSETPGTSESATPESSGGSSGSS	FLV_P10273_3mut
GSSPAPEAAK	XMRV6_A1Z651_3mut
EAAKGGSGGG	MLVFF_P26809_3mutA
EAAKEAAKEAAKEAAK	MLVFF_P26809_3mutA
GSSPAP	WMSV_P03359_3mutA
PAPAPAPAP	MLVAV_P03356_3mutA
PAPGGSEAAK	KORV_Q9TTC1_3mut
GGSGSSEAAK	MLVBM_Q7SVK7_3mutA_WS
GSSGGG	MLVCB_P08361_3mutA
GGGEAAKGGG	PERV_Q4VFZ2_3mut
PAPGGSGGG	MLVFF_P26809_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAK	FFV_O93209
PAPGGGGSS	MLVMS_P03355_3mutA_WS
EAAKGGG	MLVAV_P03356_3mutA
EAAKEAAKEAAKEAAKEAAKEAAK	MLVBM_Q7SVK7_3mutA_WS
GGSGSGSGGS	WMSV_P03359_3mutA
PAPAP	MLVMS_P03355_3mutA_WS
GSSGGGEAAK	MLVAV_P03356_3mutA
GGGGSSEAAK	MLVFF_P26809_3mutA
EAAKGGSGGS	MLVMS_P03355_PLV919
EAAKGGGGSEAAK	MLVMS_P03355_3mutA_WS
GGGGGGGG	MLVMS_P03355_PLV919
GSSGSSGSS	MLVMS_P03355_PLV919
GGGEAAKPAP	PERV_Q4VFZ2_3mutA_WS
GGGGGSGGS	MLVMS_P03355_3mutA_WS

Linker amino acid sequence	RT domain name
GGGGGGG	MLVMS_P03355_PLV919
GGS	MLVMS_P03355_PLV919
GSSGGG	MLVMS_P03355_3mutA_WS
EAAAKGGSGSS	PERV_Q4VFZ2_3mutA_WS
PAPGSSEAAK	MLVMS_P03355_PLV919
GSSEAAKPAP	MLVMS_P03355_PLV919
GGSPAPGSS	BAEVM_P10272_3mutA
GSAGSAAGSGEF	MLVCB_P08361_3mut
GGSPAPGGG	PERV_Q4VFZ2_3mut
GGGGSGGGSGGGSGGGGS	MLVMS_P03355_3mut
GSSGGSGSS	PERV_Q4VFZ2_3mutA_WS
EAAAKEAAKEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mut
GGGGSEAAKGGGGS	MLVCB_P08361_3mutA
GGSEAAKGSS	MLVAV_P03356_3mutA
EAAKGGGGSEAAK	MLVCB_P08361_3mut
EAAKEAAKEAAKEAAKEAAKEAAK	XMRV6_A1Z651_3mutA
PAPGGGEAAK	MLVMS_P03355_3mutA_WS
GSSGGGEAAK	PERV_Q4VFZ2_3mutA_WS
GSSGSS	MLVCB_P08361_3mut
PAPAPAPAPAPAP	PERV_Q4VFZ2_3mut
GGSPAPGGG	MLVFF_P26809_3mutA
GGSGSGSGSGSGGS	MLVCB_P08361_3mutA
EAAKEAAK	MLVFF_P26809_3mutA
AEEAAKEAAKEAAKEAAKEAAKEAAKEAAKEAAKEAAK	GALV_P21414_3mut
PAPAPAPAPAPAP	WMSV_P03359_3mutA
GGGEAAKGGS	KORV_Q9TTC1_3mutA
EAAKGGGPAP	KORV_Q9TTC1_3mut
PAPEAAKGSS	MLVBM_Q7SVK7_3mutA_WS
PAPEAAKGSS	FLV_P10273_3mutA
PAPGGEAAK	MLVMS_P03355_3mut
GSSPAPGGG	BAEVM_P10272_3mutA
GGGEAAKPAP	KORV_Q9TTC1-Pro_3mutA
GGGGSGGGGS	MLVMS_P03355_PLV919
GGGEAAKGSS	MLVFF_P26809_3mutA
PAPGGGGSS	MLVBM_Q7SVK7_3mutA_WS
GSSEAAK	BAEVM_P10272_3mutA
GGGGGGGG	MLVMS_P03355_PLV919
PAPGSSGGS	MLVAV_P03356_3mutA
GGGGSGGGSGGGSGGGGS	BAEVM_P10272_3mutA
PAP	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
EAAAKGSSPAP	XMRV6_A1Z651_3mutA
PAPEAAAKGGS	MLVFF_P26809_3mutA
GSSGGGEAAAK	BAEVM_P10272_3mutA
PAPAPAP	MLVMS_P03355_3mutA_WS
GSSEAAAKGGG	MLVMS_P03355_PLV919
GSSEAAAK	PERV_Q4VFZ2_3mut
GGGG	MLVMS_P03355_3mutA_WS
GGGGGS	MLVMS_P03355_3mut
GGGGSSEAAAK	PERV_Q4VFZ2_3mut
EAAAKEAAAKEAAAKEAAAKEAAAKEAAAK	SFV3L_P27401-Pro_2mutA
GSSEAAAKGGS	MLVMS_P03355_3mutA_WS
PAPGSSGGS	XMRV6_A1Z651_3mutA
GGSPAP	MLVMS_P03355_3mutA_WS
GGGGSSEAAAK	BAEVM_P10272_3mut
GSSGGSGSGSGGS	AVIRE_P03360_3mutA
PAPGSSGGS	MLVFF_P26809_3mutA
GSSPAPGGG	MLVMS_P03355_3mutA_WS
GGGGGGG	MLVMS_P03355_3mutA_WS
EAAAKGGGGGS	MLVMS_P03355_3mutA_WS
EAAAKGGSGGG	MLVMS_P03355_PLV919
GGGGSSEAAAK	XMRV6_A1Z651_3mutA
GGGGSEAAAKGGGGS	MLVBM_Q7SVK7_3mutA_WS
GSSGSS	MLVMS_P03355_PLV919
GGSGGG	MLVMS_P03355_PLV919
PAPEAAAKGGG	AVIRE_P03360_3mutA
AEEAAKEAAAKEAAAKEAAAKALEAAEAAKEAAAKEAAAKEAAAKA	FOAMV_P14350-Pro_2mutA
GGGGSGSS	PERV_Q4VFZ2_3mut
GSSGSSGSSGSSGSS	KORV_Q9TTC1-Pro_3mut
GGGGSEAAAKGGGGS	MLVMS_P03355_3mutA_WS
GGGGGSPAP	FLV_P10273_3mut
GGGEAAAK	MLVMS_P03355_3mutA_WS
GGSGSGSGSGGS	FLV_P10273_3mutA
GGG	MLVMS_P03355_PLV919
GGSPAPEAAAK	BAEVM_P10272_3mutA
EAAAKEAAAK	FLV_P10273_3mutA
GGGEAAAKPAP	BAEVM_P10272_3mutA
GGGEAAAKGGS	PERV_Q4VFZ2_3mut
GSSGGSGGS	PERV_Q4VFZ2_3mut
EAAAKGGPAP	XMRV6_A1Z651_3mutA
EAAAK	MLVBM_Q7SVK7_3mutA_WS

Linker amino acid sequence	RT domain name
PAPEAAKGGG	PERV_Q4VFZ2_3mut
EAAAKGSS	MLVCB_P08361_3mutA
GGSEAAKGGG	MLVBM_Q7SVK7_3mutA_WS
GGGGSGGGSGGGSGGGGS	XMRV6_A1Z651_3mutA
GGGGSGGGSGGGSGGGSGGGGS	BAEVM_P10272_3mut
GGGGSSPAP	PERV_Q4VFZ2_3mutA_WS
GGSGSGSGSGSGSGSGGS	PERV_Q4VFZ2_3mut
GGGEAAKPAP	PERV_Q4VFZ2_3mut
EAAAKEAAK	BAEVM_P10272_3mutA
GGSGSSEAAK	XMRV6_A1Z651_3mutA
PAPEAAKGSS	WMSV_P03359_3mutA
PAPAPAPAPAP	XMRV6_A1Z651_3mutA
GSSGGGEAAK	MLVMS_P03355_PLV919
GSSPAPGGG	MLVFF_P26809_3mutA
GGSPAPEAAK	MLVFF_P26809_3mut
PAPGSEAAK	PERV_Q4VFZ2_3mut
GGGGSS	MLVFF_P26809_3mutA
GGSGSSGGG	BAEVM_P10272_3mutA
GSSGGGEAAK	MLVMS_P03355_3mutA_WS
EAAAKGGS	MLVBM_Q7SVK7_3mutA_WS
GGGPAPGGG	MLVMS_P03355_PLV919
EAAAKEAAK	MLVMS_P03355_PLV919
GSSGSSGSS	MLVMS_P03355_PLV919
GGGEAAKPAP	MLVAV_P03356_3mutA
SGSETPGTSESATPES	FLV_P10273_3mutA
PAPAPAPAPAP	KORV_Q9TTC1-Pro_3mut
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	BAEVM_P10272_3mutA
PAPGSSGGG	MLVMS_P03355_3mutA_WS
GSSGGGEAAK	XMRV6_A1Z651_3mutA
GGGGSGGGSGGGSGGGSGGGGS	XMRV6_A1Z651_3mutA
GGGGSSPAP	MLVFF_P26809_3mutA
GGSGGPAP	PERV_Q4VFZ2_3mutA_WS
GSS	PERV_Q4VFZ2_3mut
EAAAKGSSPAP	MLVMS_P03355_3mut
EAAAKGGG	XMRV6_A1Z651_3mutA
GSSGSSGSSGSS	WMSV_P03359_3mutA
PAPEAAKGSS	MLVMS_P03355_PLV919
GSSEAAK	AVIRE_P03360_3mutA
EAAAKGGSGSS	AVIRE_P03360_3mutA
GSSEAAK	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
GGSGSSEAAK	MLVMS_P03355_PLV919
GGSEAAKGGG	MLVFF_P26809_3mutA
GGGGSGGGSGGGSGGGGS	MLVAV_P03356_3mutA
PAPAPAPAPAPAP	MLVFF_P26809_3mut
EAAAKPAPGSS	KORV_Q9TTC1-Pro_3mut
PAPGSSEAAK	MLVAV_P03356_3mutA
GGGGSSPAP	WMSV_P03359_3mutA
EAAKGGGGGS	MLVMS_P03355_3mutA_WS
GGGEAAKGGG	MLVMS_P03355_3mut
GGSGSSGGG	MLVMS_P03355_3mut
GGGPAPGGS	MLVAV_P03356_3mutA
PAPGGGGGS	MLVMS_P03355_PLV919
GGGPAPGSS	PERV_Q4VFZ2_3mut
GGGGGGG	MLVFF_P26809_3mutA
GGSGGGGSS	MLVCB_P08361_3mutA
GGGGGGG	FLV_P10273_3mutA
GGSEAAKGGSS	PERV_Q4VFZ2_3mut
GGSPAPGGG	BAEVM_P10272_3mutA
GGSPAPGSS	AVIRE_P03360_3mutA
GGSGSGSGSGGS	KORV_Q9TTC1_3mut
EAAAKEAAKEAAKEAAKEAAK	MLVBM_Q7SVK7_3mut
PAPGSSGGG	XMRV6_A1Z651_3mut
EAAKGGGGSS	PERV_Q4VFZ2_3mutA_WS
GGSGSGSGSGSGGS	PERV_Q4VFZ2_3mutA_WS
PAPGGSGGG	MLVMS_P03355_PLV919
PAPGSSGGG	PERV_Q4VFZ2_3mutA_WS
GSSGSS	BAEVM_P10272_3mutA
EAAKGGSS	MLVFF_P26809_3mutA
GGGPAP	MLVMS_P03355_PLV919
EAAKGGGGGS	MLVFF_P26809_3mutA
EAAKGGSPAP	MLVBM_Q7SVK7_3mutA_WS
EAAKEAAKEAAKEAAKEAAKEAAK	WMSV_P03359_3mutA
GSSPAPGGG	MLVBM_Q7SVK7_3mutA_WS
GGGEAAKGGSS	AVIRE_P03360_3mutA
GGGGSSEAAK	AVIRE_P03360_3mutA
GGGGGGGGG	PERV_Q4VFZ2_3mutA_WS
PAPGSSEAAK	BAEVM_P10272_3mutA
EAAKGGSS	MLVFF_P26809_3mut
GSSEAAKGGG	MLVCB_P08361_3mutA
GGSEAAK	MLVBM_Q7SVK7_3mutA_WS

Linker amino acid sequence	RT domain name
GSSEAAKGGG	PERV_Q4VFZ2_3mutA_WS
PAPGGSGGG	WMSV_P03359_3mutA
GSSGGSGGG	MLVCB_P08361_3mutA
EAAAKGSSGGG	FLV_P10273_3mutA
GSSEAAK	MLVCB_P08361_3mutA
GSSGGGEAAK	MLVMS_P03355_3mut
GGGGSGGGGS	MLVCB_P08361_3mutA
EAAAKGGGSEAAK	MLVBM_Q7SVK7_3mutA_WS
EAAAKGGG	PERV_Q4VFZ2_3mutA_WS
EAAAKGGSPAP	MLVMS_P03355_PLV919
GGGPAPGGS	AVIRE_P03360_3mutA
GSSEAAK	MLVBM_Q7SVK7_3mutA_WS
GSSGGGEAAK	PERV_Q4VFZ2_3mut
SGSETPGTSESATPES	MLVMS_P03355_PLV919
GGSGSSPAP	MLVMS_P03355_3mut
GGGGGG	MLVBM_Q7SVK7_3mutA_WS
GGSPAPGGG	XMRV6_A1Z651_3mutA
GGSGSS	PERV_Q4VFZ2_3mutA_WS
PAP	MLVBM_Q7SVK7_3mutA_WS
EAAAKPAPGSS	MLVMS_P03355_PLV919
EAAAKGGG	MLVMS_P03355_3mut
GSSEAAKPAP	PERV_Q4VFZ2_3mutA_WS
GGGGSS	MLVMS_P03355_3mutA_WS
GGSGSSEAAK	PERV_Q4VFZ2_3mut
GGGGSS	BAEVM_P10272_3mutA
PAPAP	MLVFF_P26809_3mut
PAPEAAKGGG	BAEVM_P10272_3mutA
EAAAKGGS	MLVMS_P03355_PLV919
PAPAPAPAPAPAP	PERV_Q4VFZ2_3mutA_WS
GGGGGSEAAK	MLVMS_P03355_3mut
PAPGGS	PERV_Q4VFZ2_3mut
GGGGSS	MLVCB_P08361_3mutA
GGGGSS	MLVAV_P03356_3mutA
GSSPAPEAAK	MLVMS_P03355_PLV919
GGGGSSGGG	MLVFF_P26809_3mutA
PAPEAAKGSS	MLVMS_P03355_PLV919
GGSGSSEAAK	MLVMS_P03355_3mutA_WS
EAAAKGGG	MLVAV_P03356_3mutA
PAPGSSEAAK	FLV_P10273_3mutA
EAAAKGSSGGG	MLVCB_P08361_3mutA

Linker amino acid sequence	RT domain name
PAPEAAAK	KORV_Q9TTC1-Pro_3mutA
GGSPAPEAAAK	KORV_Q9TTC1-Pro_3mut
GGSGGSGGSGGSGGSGGS	MLVAV_P03356_3mutA
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GSSGGGEAAAK	XMRV6_A1Z651_3mut
PAPGGSGGG	AVIRE_P03360_3mutA
PAPGGSEAAAK	PERV_Q4VFZ2_3mutA_WS
GGGGS	MLVMS_P03355_3mutA_WS
GGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mutA_WS
PAPAPAPAPAP	PERV_Q4VFZ2_3mutA_WS
EAAAKEAAAKEAAAKEAAKEAAAK	MLVMS_P03355_3mut
GSSGGSEAAAK	MLVMS_P03355_3mutA_WS
GGSGGSGGGSGGS	WMSV_P03359_3mutA
EAAAKGSSGGG	WMSV_P03359_3mutA
EAAAKGGG	PERV_Q4VFZ2_3mutA_WS
SGSETPGTSESATPES	PERV_Q4VFZ2_3mut
PAPGSSGGS	MLVMS_P03355_3mutA_WS
PAPEAAAKGSS	PERV_Q4VFZ2_3mut
PAPEAAAK	AVIRE_P03360_3mutA
GSSEAAAKGGG	BAEVM_P10272_3mutA
GSSPAP	MLVAV_P03356_3mutA
EAAAKEAAAKEAAAKEAAAK	MLVFF_P26809_3mut
PAPGGSGSS	MLVAV_P03356_3mutA
GGGSGGGGSGGGGS	PERV_Q4VFZ2_3mutA_WS
GSSGGSEAAAK	MLVCB_P08361_3mutA
EAAAKGGS	KORV_Q9TTC1-Pro_3mutA
EAAAKGGS	MLVFF_P26809_3mutA
GGSPAP	MLVMS_P03355_PLV919
GGSGSS	MLVMS_P03355_PLV919
SGSETPGTSESATPES	WMSV_P03359_3mut
GGGGGGG	WMSV_P03359_3mut
GGSPAPGSS	MLVCB_P08361_3mutA
GGGSGSGGS	WMSV_P03359_3mut
PAPGGS	MLVMS_P03355_PLV919
PAPGSSGGS	MLVCB_P08361_3mutA
EAAAKEAAAKEAAAKEAAKEAAAK	MLVFF_P26809_3mut
SGGSSGGSSGSETPGTSESATPESGSSGGSS	PERV_Q4VFZ2_3mut
GGSGGSGGSGGSGGS	BAEVM_P10272_3mutA
GSSEAAAK	PERV_Q4VFZ2_3mut

Linker amino acid sequence	RT domain name
EAAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mutA
GGSGSGSGSGSGSGS	MLVMS_P03355_3mut
PAPAPAPAPAPAP	MLVMS_P03355_3mut
GGSPAPEAAK	MLVMS_P03355_PLV919
EAAK	WMSV_P03359_3mutA
EAAKGSSGGS	MLVBM_Q7SVK7_3mutA_WS
GGSGGGGSS	MLVMS_P03355_3mutA_WS
GGGEAAKPAP	MLVMS_P03355_3mut
EAAKGSGGG	XMRV6_A1Z651_3mutA
GGGGGSEAAK	KORV_Q9TTC1-Pro_3mutA
GGGGGG	BAEVM_P10272_3mutA
GGGGGG	MLVMS_P03355_3mut
GGGGGG	MLVBM_Q7SVK7_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKA	AVIRE_P03360
PAPGSGGS	PERV_Q4VFZ2_3mut
GGGGGS	XMRV6_A1Z651_3mut
EAAKPAP	XMRV6_A1Z651_3mutA
GGG	MLVMS_P03355_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKA	FLV_P10273_3mut
EAAKGSSPAP	MLVMS_P03355_3mut
SGSETPGTSESATPES	BAEVM_P10272_3mutA
GGSPAPEAAK	MLVMS_P03355_3mut
GSSGSSGSSGSS	MLVAV_P03356_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKA	MLVMS_P03355_3mut
GGSPAP	MLVCB_P08361_3mutA
GGGGGSEAAK	MLVMS_P03355_3mutA_WS
GGGGG	MLVFF_P26809_3mutA
GSSEAAK	MLVAV_P03356_3mutA
GGS	BAEVM_P10272_3mut
EAAKGSPAP	MLVCB_P08361_3mutA
PAPAPAPAP	FLV_P10273_3mutA
PAPGGGEAAK	MLVCB_P08361_3mutA
GGGGSSEAAK	MLVMS_P03355_3mutA_WS
GGGGG	PERV_Q4VFZ2_3mutA_WS
GGSGSGSGSGSGSGS	PERV_Q4VFZ2_3mut
GGGGG	MLVMS_P03355_3mut
PAPEAAKGGG	MLVBM_Q7SVK7_3mutA_WS
GSSGGGPAP	XMRV6_A1Z651_3mutA
GSSGSSGSSGSSGSS	PERV_Q4VFZ2_3mutA_WS
EAAKGSPAP	PERV_Q4VFZ2_3mut

Linker amino acid sequence	RT domain name
GSSGGSEAAAK	MLVMS_P03355_PLV919
GSS	PERV_Q4VFZ2_3mut
EAAAKGGS	WMSV_P03359_3mutA
GGGGGSPAP	PERV_Q4VFZ2_3mutA_WS
EAAAKGSS	MLVMS_P03355_PLV919
EAAAKGGGGSS	KORV_Q9TTC1-Pro_3mutA
PAPGSSGGG	PERV_Q4VFZ2_3mut
GGGGSSEAAAK	MLVFF_P26809_3mut
PAPAPAP	MLVMS_P03355_3mut
GSSGGSEAAAK	XMRV6_A1Z651_3mut
PAPEAAKGSS	MLVMS_P03355_3mutA_WS
GGSGSGSGSGSGGS	MLVMS_P03355_3mutA_WS
GGSGSSPAP	XMRV6_A1Z651_3mutA
GGGGSSPAP	MLVMS_P03355_PLV919
GGGGS	MLVCB_P08361_3mutA
EAAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
EAAAKEAAK	KORV_Q9TTC1_3mutA
PAPGGGEAAAK	BAEVM_P10272_3mutA
GSSGGSEAAAK	XMRV6_A1Z651_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	FLV_P10273_3mut
GSSEAAKPAP	MLVMS_P03355_3mutA_WS
EAAAKPAPGSS	PERV_Q4VFZ2_3mutA_WS
GSSGGSPAP	XMRV6_A1Z651_3mutA
GSSEAAKGGG	PERV_Q4VFZ2_3mut
GGGEAAKGGG	WMSV_P03359_3mutA
GSSEAAKGGG	MLVFF_P26809_3mut
PAPAPAP	KORV_Q9TTC1-Pro_3mutA
EAAAKGGSPAP	MLVMS_P03355_3mutA_WS
PAPGGSEAAAK	PERV_Q4VFZ2_3mut
GGGGS	MLVBM_Q7SVK7_3mutA_WS
EAAAKGSSGGG	KORV_Q9TTC1_3mut
EAAAKGGGPAP	MLVCB_P08361_3mutA
EAAAKGSS	BAEVM_P10272_3mutA
GGSPAPGGG	MLVBM_Q7SVK7_3mutA_WS
GGGGSEAAKGGGGS	MLVMS_P03355_3mutA_WS
GGGEAAKGGG	PERV_Q4VFZ2_3mutA_WS
EAAAKGGGGSS	MLVMS_P03355_3mutA_WS
EAAAKGGGPAP	MLVFF_P26809_3mut
GSSPAP	PERV_Q4VFZ2_3mutA_WS
EAAAKGGS	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
GGGGSS	KORV_Q9TTC1-Pro_3mutA
EAAAKGSSPAP	MLVMS_P03355_3mutA_WS
GGGPAP	PERV_Q4VFZ2_3mut
EAAAKGSSGGS	XMRV6_A1Z651_3mutA
PAPGGG	MLVAV_P03356_3mutA
GSSPAPEAAK	BAEVM_P10272_3mutA
GGGPAP	MLVBM_Q7SVK7_3mutA_WS
GSSGGGGGS	AVIRE_P03360_3mutA
SGSETPGTSESATPES	MLVMS_P03355_PLV919
GGGPAP	MLVFF_P26809_3mut
EAAAKGGGSS	XMRV6_A1Z651_3mutA
GGGGSSPAP	XMRV6_A1Z651_3mut
GGGGSEAAKGGGGS	MLVMS_P03355_3mut
GSSPAP	MLVBM_Q7SVK7_3mutA_WS
GSSGSEAAK	FLV_P10273_3mutA
SGSETPGTSESATPES	MLVBM_Q7SVK7_3mutA_WS
PAPGGG	AVIRE_P03360_3mutA
GGGEAAKPAP	MLVMS_P03355_3mutA_WS
EAAAKGGSGSS	PERV_Q4VFZ2_3mut
GSSPAPGGG	MLVAV_P03356_3mutA
PAPGGSGSS	BAEVM_P10272_3mutA
GSSGGSPAP	MLVFF_P26809_3mutA
EAAAKSSGGG	PERV_Q4VFZ2_3mut
GGGGSGGGGS	PERV_Q4VFZ2_3mutA_WS
GSSGGGGGS	BAEVM_P10272_3mutA
GGGGSGGGS	MLVBM_Q7SVK7_3mutA_WS
EAAAKGGS	PERV_Q4VFZ2_3mutA_WS
GSSGSSGSSGSS	MLVMS_P03355_3mut
GGS	MLVMS_P03355_3mutA_WS
GSSGSEAAK	MLVBM_Q7SVK7_3mutA_WS
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	XMRV6_A1Z651
GGGGG	FLV_P10273_3mutA
PAPEAAKGSS	PERV_Q4VFZ2_3mut
GGGGGG	WMSV_P03359_3mut
EAAAKGGG	BAEVM_P10272_3mutA
GGGGSS	MLVMS_P03355_3mutA_WS
GSSGGGEAAK	KORV_Q9TTC1_3mut
GSSGSS	AVIRE_P03360_3mutA
EAAKPAP	MLVMS_P03355_3mut
EAAKEAAKEAAK	FLV_P10273_3mutA

Linker amino acid sequence	RT domain name
GGGG	XMRV6_A1Z651_3mutA
GSSPAPGGS	BAEVM_P10272_3mutA
GSSGGGGGS	MLVFF_P26809_3mutA
GGGGSSGGGS	MLVAV_P03356_3mutA
GGS	PERV_Q4VFZ2_3mut
GGGGG	WMSV_P03359_3mutA
GSSGSSGSSGSSGSSGSS	FLV_P10273_3mutA
PAPGGGGSS	MLVAV_P03356_3mutA
GGGGGGGG	BAEVM_P10272_3mutA
SGSETPGTSESATPES	MLVCB_P08361_3mutA
PAPGGG	BAEVM_P10272_3mutA
GSSGSSGSS	MLVCB_P08361_3mutA
GGSGSS	MLVMS_P03355_3mutA_WS
EAAAKGGGGSEAAAK	WMSV_P03359_3mutA
GGGGGGGG	FLV_P10273_3mutA
GSSGSS	MLVMS_P03355_3mutA_WS
PAPEAAAKGGS	XMRV6_A1Z651_3mutA
EAAKEAAK	MLVMS_P03355_3mut
GGGGSGGGSGGGGS	BAEVM_P10272_3mutA
EAAKGSSPAP	MLVMS_P03355_PLV919
GGGGSSEAAK	MLVMS_P03355_3mut
GGGGSSEAAK	BAEVM_P10272_3mutA
PAPGGSGSS	PERV_Q4VFZ2_3mut
GGSGGGEAAK	MLVFF_P26809_3mut
PAPEAAAKGGS	PERV_Q4VFZ2_3mut
GGGPAPGSS	AVIRE_P03360_3mut
PAPGGSGGG	PERV_Q4VFZ2_3mutA_WS
GGGGGGGG	PERV_Q4VFZ2_3mutA_WS
GSSEAAK	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGGS	PERV_Q4VFZ2_3mutA_WS
EAAKGGS	MLVMS_P03355_3mut
GGGGSGSS	MLVCB_P08361_3mut
GGGPAP	KORV_Q9TTC1-Pro_3mutA
EAAKPAPGGG	MLVCB_P08361_3mut
GSSGSPAP	MLVCB_P08361_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVMS_P03355_3mut
PAPAPAPAP	MLVMS_P03355_3mut
GSSGGS	XMRV6_A1Z651_3mutA
GSSEAAKGGG	MLVMS_P03355_3mut
GGSGSSPAP	MLVMS_P03355_3mutA_WS

Linker amino acid sequence	RT domain name
GSSEAAKGG	MLVMS_P03355_PLV919
EAAAKEAAKEAAKEAAKEAAK	BAEVM_P10272_3mut
PAPGGGGSS	KORV_Q9TTC1_3mutA
EAAKGGSS	MLVMS_P03355_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	FFV_O93209_2mut
GGSGGGGGSGGGSGGGSS	BAEVM_P10272_3mutA
GGGGGG	MLVMS_P03355_PLV919
PAPEAAK	BAEVM_P10272_3mutA
GGSGSSEAAK	MLVAV_P03356_3mutA
GGG	MLVCB_P08361_3mutA
GGGGG	MLVCB_P08361_3mutA
GGSGGGGGSGGGSS	KORV_Q9TTC1-Pro_3mutA
GSSGSSGSSGSSGSSGSS	XMRV6_A1Z651_3mutA
GSSEAAKPAP	FLV_P10273_3mutA
GGGEAAKPAP	MLVCB_P08361_3mutA
GSSGSSGSS	MLVMS_P03355_3mutA_WS
PAPAPAPAP	MLVMS_P03355_PLV919
EAAKGGG	MLVMS_P03355_PLV919
PAPAPAPAPAPAP	FLV_P10273_3mutA
EAAKGGSGSS	MLVMS_P03355_3mut
GGGGGG	PERV_Q4VFZ2_3mutA_WS
PAPGGG	MLVCB_P08361_3mutA
GGGGGGSGSS	KORV_Q9TTC1_3mutA
GGGGSGGGGGSGGGSGGGSS	XMRV6_A1Z651_3mut
GGSGGGSS	KORV_Q9TTC1-Pro_3mutA
EAAKPAPGGG	MLVMS_P03355_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	XMRV6_A1Z651
GGGGSGGGGGSGGGSGGGSGGGSS	FLV_P10273_3mutA
EAAKGGGGSEAAK	PERV_Q4VFZ2_3mutA_WS
GGGPAPGSS	AVIRE_P03360_3mutA
GGGGG	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGSGGGSGGGSGGGSS	MLVMS_P03355_3mut
GGGGSGGGSS	MLVMS_P03355_3mutA_WS
EAAKGGSPAP	XMRV6_A1Z651_3mutA
EAAKGSSPAP	AVIRE_P03360_3mutA
PAPGGSGSS	KORV_Q9TTC1-Pro_3mutA
GSS	MLVBM_Q7SVK7_3mutA_WS
GSS	WMSV_P03359_3mut
GGGPAPGSS	MLVFF_P26809_3mutA
EAAKPAP	MLVMS_P03355_3mut

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Linker amino acid sequence	RT domain name
GGGPAPGSS	MLVMS_P03355_3mutA_WS
GGGGSS	KORV_Q9TTC1_3mutA
GSSGGSGGG	BAEVM_P10272_3mutA
EAAAKEAAKEAAK	MLVCB_P08361_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	FLV_P10273_3mutA
PAPGGGGSS	PERV_Q4VFZ2_3mut
PAPAPAPAPAP	KORV_Q9TTC1-Pro_3mutA
EAAAK	MLVMS_P03355_3mutA_WS
GGG	MLVCB_P08361_3mut
GGSEAAKGGG	BAEVM_P10272_3mutA
GGGGGGSS	MLVAV_P03356_3mutA
EAAAKGSSPAP	MLVBM_Q7SVK7_3mutA_WS
GGSGGGSS	XMRV6_A1Z651_3mut
EAAAKPAPGGG	KORV_Q9TTC1-Pro_3mutA
GGGPAPEAAK	FLV_P10273_3mutA
GGSPAPEAAK	MLVMS_P03355_3mutA_WS
GGSGGGSGSGSGSS	MLVFF_P26809_3mut
EAAKGGSGSS	MLVMS_P03355_PLV919
GGGEAAKGGSS	MLVBM_Q7SVK7_3mutA_WS
PAPAPAPAP	BAEVM_P10272_3mutA
EAAAKEAAKEAAKEAAK	MLVMS_P03355_3mut
EAAAKPAP	XMRV6_A1Z651_3mut
EAAKEAAK	MLVBM_Q7SVK7_3mutA_WS
EAAKGGG	BAEVM_P10272_3mut
EAAKGGSS	MLVAV_P03356_3mutA
EAAKEAAKEAAKEAAKEAAKEAAK	MLVFF_P26809_3mut
GGGPAPGSS	PERV_Q4VFZ2_3mutA_WS
GGGG	PERV_Q4VFZ2_3mut
EAAKGGSGSS	MLVMS_P03355_PLV919
GGGGSGGGSGGGSS	MLVMS_P03355_3mutA_WS
EAAAK	MLVMS_P03355_3mutA_WS
GGGGSS	PERV_Q4VFZ2
PAPEAAKGGSS	MLVCB_P08361_3mut
GSS	MLVMS_P03355_3mut
GSAGSAAGSGEF	MLVFF_P26809_3mutA
EAAKEAAKEAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mut
GGGGSGGGSS	AVIRE_P03360_3mutA
EAAAK	MLVMS_P03355_3mut
GGGPAPGSS	PERV_Q4VFZ2_3mut
GGGGSGGGSGGGSS	MLVMS_P03355_PLV919

Linker amino acid sequence	RT domain name
PAPGGG	MLVMS_P03355_3mutA_WS
GGGEAAKPAP	PERV_Q4VFZ2_3mutA_WS
EAAAKPAPGSS	KORV_Q9TTC1-Pro_3mutA
PAPGSS	KORV_Q9TTC1_3mutA
GSAGSAAGSGEF	PERV_Q4VFZ2_3mut
PAPGGGGSS	KORV_Q9TTC1-Pro_3mutA
GSSGGGEAAK	MLVCB_P08361_3mutA
GSS	AVIRE_P03360_3mutA
GSSGSSGSSGSS	XMRV6_A1Z651_3mutA
PAPEAAKGGG	MLVMS_P03355_PLV919
GGGPAPEAAK	MLVCB_P08361_3mutA
PAPGGGGSS	MLVCB_P08361_3mutA
EAAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
GGGGGSPAP	MLVFF_P26809_3mutA
GSSGSSGSSGSSGSS	PERV_Q4VFZ2
GSSPAPEAAK	MLVMS_P03355_PLV919
GSSGSSGSSGSSGSSGSS	MLVBM_Q7SVK7_3mutA_WS
GSSGSSGSSGSSGSSGSS	MLVMS_P03355_3mutA_WS
GGSPAPEAAK	MLVAV_P03356_3mutA
GSSGGG	BAEVM_P10272_3mut
EAAKGSSGSS	KORV_Q9TTC1-Pro_3mutA
GGSGSSEAAK	MLVMS_P03355_3mutA_WS
GGGPAPEAAK	MLVFF_P26809_3mutA
GGGPAPGSS	MLVMS_P03355_3mutA_WS
GGGGG	MLVMS_P03355_PLV919
GGGEAAKPAP	MLVBM_Q7SVK7_3mutA_WS
GGGGSGGGSS	WMSV_P03359_3mut
GGGPAPEAAK	PERV_Q4VFZ2_3mut
GGSGSSEAAK	MLVMS_P03355_PLV919
EAAKGGPAP	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSSGSS	KORV_Q9TTC1-Pro_3mutA
PAPAP	WMSV_P03359_3mutA
GGSPAPGSS	MLVAV_P03356_3mutA
GGSGGGPAP	MLVMS_P03355_3mut
GGSPAP	MLVMS_P03355_PLV919
EAAKGSPAP	PERV_Q4VFZ2_3mut
GSSPAPGGG	KORV_Q9TTC1-Pro_3mutA
GSAGSAAGSGEF	MLVMS_P03355_3mut
GGSPAP	PERV_Q4VFZ2_3mut
GSSGSS	KORV_Q9TTC1-Pro_3mut

Linker amino acid sequence	RT domain name
GGGPAPGSS	MLVMS_P03355_3mutA_WS
AEAAAKEAAKEAAKEAAKALEAEAAKEAAKEAAKEAAKA	FOAMV_P14350
PAPGSSGGG	MLVMS_P03355_PLV919
GGSEAAKPAP	BAEVM_P10272_3mutA
GGGGGS	MLVCB_P08361_3mutA
PAPEAAKGGS	MLVMS_P03355_3mutA_WS
EAAAKEAAKEAAKEAAKEAAKEAAK	BAEVM_P10272_3mutA
GGSEAAK	BAEVM_P10272_3mutA
GSSPAPEAAK	MLVMS_P03355_3mutA_WS
PAPGGG	WMSV_P03359_3mut
EAAKPAP	PERV_Q4VFZ2_3mut
GSSGSSGSSGSSGSS	WMSV_P03359_3mut
PAPGGG	MLVBM_Q7SVK7_3mutA_WS
GGSGGGEAAK	BAEVM_P10272_3mutA
PAPGGS	MLVMS_P03355_3mut
GGSGGSGGSGGS	MLVBM_Q7SVK7_3mutA_WS
EAAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mut
GGSEAAKGGG	WMSV_P03359_3mutA
GGGPAP	BAEVM_P10272_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGSGGGGS	XMRV6_A1Z651_3mut
GGSPAPGSS	KORV_Q9TTC1_3mut
GGGPAPGSS	MLVMS_P03355_3mut
GGGGSSGGS	BAEVM_P10272_3mutA
GGGEAAKGGS	KORV_Q9TTC1-Pro_3mutA
PAPAP	MLVBM_Q7SVK7_3mutA_WS
GGSPAPGGG	PERV_Q4VFZ2_3mut
PAPGSS	PERV_Q4VFZ2_3mutA_WS
GSSGGSPAP	MLVBM_Q7SVK7_3mutA_WS
EAAKGGGSEAAK	PERV_Q4VFZ2_3mut
GSSEAAKGGS	KORV_Q9TTC1-Pro_3mut
PAPAPAPAP	KORV_Q9TTC1-Pro_3mutA
GGSEAAKPAP	WMSV_P03359_3mutA
PAPGGS	FLV_P10273_3mutA
EAAKGGGPAP	PERV_Q4VFZ2_3mut
GGSGSSGGG	AVIRE_P03360_3mutA
EAAKGGSGSS	BAEVM_P10272_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVCB_P08361_3mutA
GSSEAAKGGS	XMRV6_A1Z651_3mutA
GGGGG	BAEVM_P10272_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	SFV3L_P27401_2mutA

Linker amino acid sequence	RT domain name
GGGEAAKGSS	MLVMS_P03355_PLV919
EAAAKGGGSEAAK	KORV_Q9TTC1_3mutA
EAAAKGGG	AVIRE_P03360_3mut
GGSGGG	MLVMS_P03355_3mutA_WS
GGSGSSGGG	MLVMS_P03355_PLV919
GGGGSGGGSGGGSGGGSGGGSGGGG	KORV_Q9TTC1_3mut
GGGGSEAAKGGGGS	KORV_Q9TTC1_3mutA
PAPAPAPAPAP	FLV_P10273_3mutA
GGS	MLVBM_Q7SVK7_3mutA_WS
GGGGSEAAK	MLVBM_Q7SVK7_3mutA_WS
GSSGSSGSSGSSGSS	MLVMS_P03355_3mutA_WS
EAAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_3mut
GGSGSSGGG	PERV_Q4VFZ2_3mut
PAP	MLVFF_P26809_3mut
GSSPAPEAAK	MLVAV_P03356_3mutA
EAAAKGGGSS	MLVMS_P03355_3mut
GGGEAAKGGS	XMRV6_A1Z651_3mut
GGSGGGPAP	MLVBM_Q7SVK7_3mutA_WS
GSAGSAAGSGEF	BAEVM_P10272_3mutA
GSSEAAK	MLVCB_P08361_3mut
PAPGSS	MLVMS_P03355_3mut
EAAAKEAAKEAAK	MLVAV_P03356_3mutA
GSAGSAAGSGEF	XMRV6_A1Z651_3mutA
GSSGSSGSSGSS	BAEVM_P10272_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mut
GGGGSSEAAK	WMSV_P03359_3mut
GSSGGGEAAK	MLVBM_Q7SVK7_3mutA_WS
EAAKPAP	MLVFF_P26809_3mutA
GGSPAPGGG	KORV_Q9TTC1_3mutA
PAPEAAK	FLV_P10273_3mutA
GSSGSSGSS	MLVBM_Q7SVK7_3mutA_WS
GSSGGGEAAK	FLV_P10273_3mutA
GGSPAP	MLVBM_Q7SVK7_3mutA_WS
GSAGSAAGSGEF	KORV_Q9TTC1-Pro_3mutA
PAPGGSEAAK	MLVMS_P03355_PLV919
GGSPAPEAAK	MLVBM_Q7SVK7_3mutA_WS
GGGGSPAP	MLVBM_Q7SVK7_3mutA_WS
EAAKGSSPAP	WMSV_P03359_3mut
EAAKGGGPAP	MLVBM_Q7SVK7_3mutA_WS
PAPGSS	KORV_Q9TTC1-Pro_3mutA

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271

Linker amino acid sequence	RT domain name
GGSGSSEAAK	PERV_Q4VFZ2_3mutA_WS
EAAAKGSS	MLVMS_P03355_3mut
GGSGSSEAAK	BAEVM_P10272_3mut
GGSGGG	MLVBM_Q7SVK7_3mutA_WS
GGGPAP	MLVMS_P03355_PLV919
GGSPAPGGG	PERV_Q4VFZ2_3mutA_WS
GGGGSEAAK	MLVFF_P26809_3mutA
EAAAKGSSGSS	MLVBM_Q7SVK7_3mut
PAPAP	XMRV6_A1Z651_3mut
GSSPAPGSS	MLVBM_Q7SVK7_3mutA_WS
GSSEAAKGGG	WMSV_P03359_3mutA
EAAAKGGGGSS	PERV_Q4VFZ2_3mut
GSSGSSGSSGSSGSS	MLVCB_P08361_3mutA
EAAAKGGGGSS	PERV_Q4VFZ2_3mut
EAAAKGSS	PERV_Q4VFZ2_3mut
EAAAKEAAKEAAKEAAKEAAKEAAK	AVIRE_P03360_3mutA
EAAAKGSS	MLVCB_P08361_3mut
GSSGSEAAK	MLVAV_P03356_3mutA
EAAAKPAPGSS	PERV_Q4VFZ2_3mut
GGSGGS	MLVAV_P03356_3mutA
EAAAKGSSGGG	AVIRE_P03360_3mutA
GGSGSGSGSGSS	PERV_Q4VFZ2_3mut
GGGGGGGG	KORV_Q9TTC1_3mutA
GGSGSSEAAK	MLVCB_P08361_3mutA
EAAAKGGG	MLVBM_Q7SVK7_3mutA_WS
GGGGSGGGSGGGSS	MLVCB_P08361_3mut
GGSGSGSGSGSS	PERV_Q4VFZ2_3mutA_WS
PAPAPAPAPAP	WMSV_P03359_3mut
EAAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mut
GGSGSGGS	XMRV6_A1Z651_3mutA
PAPGGGGSS	BAEVM_P10272_3mutA
GSSEAAKGS	MLVCB_P08361_3mut
GSSGGGPAP	MLVCB_P08361_3mutA
GGSGSS	MLVBM_Q7SVK7_3mutA_WS
GGGGSEAAK	MLVAV_P03356_3mutA
GSSEAAK	PERV_Q4VFZ2_3mutA_WS
GGGGSGSS	MLVBM_Q7SVK7_3mutA_WS
EAAAKGGSGSS	MLVFF_P26809_3mut
PAP	FLV_P10273_3mutA
GGGGG	MLVMS_P03355_3mutA_WS

Linker amino acid sequence	RT domain name
EAAAK	PERV_Q4VFZ2_3mut
GSS	FLV_P10273_3mutA
PAPAPAPAPAP	KORV_Q9TTC1-Pro_3mutA
EAAAKEAAKEAAKEAAK	MLVCB_P08361_3mut
EAAKGGGSEAAK	XMRV6_A1Z651_3mut
PAPGGSGGG	MLVBM_Q7SVK7_3mutA_WS
GGSGGSPAP	WMSV_P03359_3mutA
GGGGSSEAAK	MLVBM_Q7SVK7_3mutA_WS
PAPGGGGSS	MLVCB_P08361_3mut
GGSGSGSGSGSS	PERV_Q4VFZ2_3mutA_WS
PAPGGSGGG	MLVMS_P03355_3mutA_WS
GSSPAPGGS	MLVCB_P08361_3mutA
GSSGSSGSS	MLVFF_P26809_3mut
PAPGGGGSS	MLVBM_Q7SVK7_3mutA_WS
GSSPAP	PERV_Q4VFZ2_3mut
GGSGGG	KORV_Q9TTC1-Pro_3mut
EAAKGGGSEAAK	PERV_Q4VFZ2_3mutA_WS
GGSPAPEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKPAP	BAEVM_P10272_3mut
GGGSGGGSGGGSGGGSGGGSGGGGS	MLVMS_P03355_3mut
EAAKGGGGSS	MLVFF_P26809_3mut
EAAKEAAK	MLVCB_P08361_3mut
GSSEAAKGGS	PERV_Q4VFZ2_3mut
GGSPAP	KORV_Q9TTC1-Pro_3mutA
EAAKEAAKEAAKEAAK	MLVMS_P03355_3mutA_WS
GSSGSSGSSGSSGSS	BAEVM_P10272_3mut
PAPEAAK	MLVMS_P03355_3mut
GSSGSPAP	PERV_Q4VFZ2
GGGPAPGGS	BAEVM_P10272_3mutA
EAAKPAPGGS	MLVMS_P03355_PLV919
GGGGSGGGGS	PERV_Q4VFZ2
GGGEAAK	KORV_Q9TTC1-Pro_3mut
EAAKGGGGGS	FLV_P10273_3mutA
GSSPAPGSS	MLVMS_P03355_3mut
GSSPAPEAAK	MLVMS_P03355_3mutA_WS
GSAGSAAGSGEF	MLVBM_Q7SVK7_3mutA_WS
EAAAK	BAEVM_P10272_3mutA
EAAKGGGGSS	BAEVM_P10272_3mutA
GGG	WMSV_P03359_3mut
GGSGSSPAP	BAEVM_P10272_3mut

Linker amino acid sequence	RT domain name
GGSEAAKPAP	MLVBM_Q7SVK7_3mutA_WS
EAAKGGSGSS	MLVCB_P08361_3mut
PAPGSS	MLVAV_P03356_3mutA
PAPEAAKGGG	MLVCB_P08361_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	FOAMV_P14350-Pro_2mut
GSSGSSGSS	PERV_Q4VFZ2_3mut
PAPGGG	MLVMS_P03355_3mut
PAPGSS	PERV_Q4VFZ2_3mut
GSSGGG	MLVMS_P03355_PLV919
GSSGSSGSSGSSGSSGSS	WMSV_P03359_3mut
PAP	AVIRE_P03360_3mutA
EAAKGGSPAP	MLVBM_Q7SVK7_3mutA_WS
GSSGSSGSSGSS	MLVMS_P03355_PLV919
GGGGSGGGSGGGSGGGSGGGGS	AVIRE_P03360
GGGGG	PERV_Q4VFZ2_3mut
EAAKGGSGGG	MLVBM_Q7SVK7_3mutA_WS
GGGGGG	KORV_Q9TTC1-Pro_3mut
GGSGSEAAK	PERV_Q4VFZ2_3mut
GSSPAPEAAK	MLVBM_Q7SVK7_3mutA_WS
GGGGSGGGGS	MLVBM_Q7SVK7_3mutA_WS
GSSGGGGGS	MLVAV_P03356_3mutA
GSAGSAAGSGEF	WMSV_P03359_3mutA
GGGEAAKGSS	BAEVM_P10272_3mutA
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	FFV_O93209-Pro_2mut
PAPGGSGGG	MLVCB_P08361_3mutA
EAAKEAAKEAAKEAAKEAAK	SFV3L_P27401_2mut
GGSGSPAP	MLVMS_P03355_PLV919
GGGGGG	PERV_Q4VFZ2_3mut
EAAKEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mut
EAAKGGSPAP	MLVFF_P26809_3mut
GGGPAPGSS	MLVBM_Q7SVK7_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	SFV3L_P27401
PAP	PERV_Q4VFZ2_3mut
EAAKGGG	MLVMS_P03355_PLV919
GSSGSEAAK	WMSV_P03359_3mutA
GGSGSEAAK	KORV_Q9TTC1-Pro_3mutA
EAAKEAAKEAAK	PERV_Q4VFZ2
GGSGGGEAAK	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGSGGGGS	BAEVM_P10272_3mut
EAAKGGG	XMRV6_A1Z651_3mutA

Linker amino acid sequence	RT domain name
GSSGGGGGS	WMSV_P03359_3mutA
GSSGSSGSSGSSGSSGSS	MLVFF_P26809_3mutA
GSSGSS	MLVAV_P03356_3mutA
EAAAKGGGSEAAAK	MLVMS_P03355_PLV919
EAAAKGGGPAP	PERV_Q4VFZ2
GGSEAAKGGG	MLVAV_P03356_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	MLVBM_Q7SVK7_3mut
EAAKEAAKEAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mutA
GSSPAPEAAK	MLVFF_P26809_3mutA
GGGSEAAKGGGGS	PERV_Q4VFZ2_3mut
GSSGSSGSSGSS	PERV_Q4VFZ2_3mut
GGSEAAK	MLVFF_P26809_3mutA
GGGGGGGG	MLVMS_P03355_3mut
GSSGGG	XMRV6_A1Z651_3mutA
EAAAKGGS	BAEVM_P10272_3mutA
GGGGS	BAEVM_P10272_3mutA
GGSEAAKGGG	KORV_Q9TTC1-Pro_3mutA
GGSGSSGGG	KORV_Q9TTC1_3mutA
GGSGSSEAAK	WMSV_P03359_3mut
EAAAKGGSGSS	MLVBM_Q7SVK7_3mutA_WS
GGS	BAEVM_P10272_3mutA
GGGPAPGSS	WMSV_P03359_3mutA
GSSGSSGSSGSSGSS	AVIRE_P03360_3mut
GGGEAAKAP	XMRV6_A1Z651_3mut
GSSGGG	MLVFF_P26809_3mutA
GGSPAPGSS	PERV_Q4VFZ2_3mut
PAPGGS	MLVCB_P08361_3mut
PAPAPAPAP	KORV_Q9TTC1_3mutA
GSSGGS	MLVCB_P08361_3mutA
GSSGGSEAAK	PERV_Q4VFZ2_3mut
EAAAKGSSGGS	MLVMS_P03355_PLV919
EAAAKGGG	WMSV_P03359_3mut
PAPGGGGGS	BAEVM_P10272_3mutA
GGGGSEAAKGGGGS	WMSV_P03359_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_3mutA_WS
GGS	KORV_Q9TTC1-Pro_3mutA
GSSGGSPAP	BAEVM_P10272_3mutA
GGG	MLVMS_P03355_PLV919
PAPGSS	KORV_Q9TTC1-Pro_3mut
GGSEAAKGGG	FLV_P10273_3mutA

Linker amino acid sequence	RT domain name
GGSEAAKPAP	PERV_Q4VFZ2_3mutA_WS
GGGGSSPAP	XMRV6_A1Z651_3mutA
EAAAKEAAKEAAKEAAKEAAK	PERV_Q4VFZ2_3mutA_WS
GGGG	PERV_Q4VFZ2_3mutA_WS
GGSEAAKPAP	MLVMS_P03355_3mut
PAPGSSGGG	MLVMS_P03355_3mutA_WS
PAPEAAKGGG	AVIRE_P03360_3mut
GGGGSSPAP	MLVMS_P03355_3mutA_WS
GGGGSGGGSGGGSGGGG	PERV_Q4VFZ2_3mut
GGGEAAK	MLVMS_P03355_3mut
GGGGSS	MLVFF_P26809_3mut
GGSPAPGSS	XMRV6_A1Z651_3mut
GGGGG	KORV_Q9TTC1-Pro_3mutA
EAAAKGSSGGG	FLV_P10273_3mutA
GSS	MLVMS_P03355_PLV919
GGGG	MLVMS_P03355_PLV919
GSSGGG	MLVMS_P03355_PLV919
GGSGGGGGSGGG	MLVMS_P03355_3mut
PAPEAAKGGG	MLVMS_P03355_3mut
EAAAKGSSGGG	BAEVM_P10272_3mutA
GSSEAAK	KORV_Q9TTC1-Pro_3mutA
GSAGSAAGSGEF	KORV_Q9TTC1_3mutA
GGGGGSEAAK	MLVCB_P08361_3mut
GGGG	WMSV_P03359_3mut
GGGGSSEAAK	MLVMS_P03355_PLV919
PAPGGG	WMSV_P03359_3mutA
EAAAKGSGGG	MLVAV_P03356_3mutA
GGGPAPGGS	MLVMS_P03355_3mut
EAAKPAP	PERV_Q4VFZ2_3mutA_WS
GSSGSSGSS	KORV_Q9TTC1-Pro_3mutA
GSSPAPGGS	XMRV6_A1Z651_3mut
GGGGSPAP	BAEVM_P10272_3mutA
GGSGSSGGG	PERV_Q4VFZ2_3mutA_WS
GGGEAAKGGG	AVIRE_P03360_3mut
GSSEAAK	FLV_P10273_3mutA
EAAK	MLVMS_P03355_3mut
EAAAKGSGSS	WMSV_P03359_3mut
GSSEAAKGGG	PERV_Q4VFZ2_3mut
PAPGSSGGG	BAEVM_P10272_3mutA
EAAKGGGGG	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
GGSEAAKPAP	AVIRE_P03360_3mut
GGGPAPGGS	XMRV6_A1Z651_3mut
GGGGS	KORV_Q9TTC1_3mutA
GGSGSGSGSGSGGS	XMRV6_A1Z651_3mut
GGGPAP	KORV_Q9TTC1-Pro_3mut
EAAKPAP	MLVBM_Q7SVK7_3mutA_WS
GGSEAAK	MLVMS_P03355_PLV919
GSSEAAKPAP	KORV_Q9TTC1-Pro_3mutA
GGSGSS	MLVMS_P03355_3mut
EAAKPAPGGG	PERV_Q4VFZ2_3mut
GGSPAPEAAK	KORV_Q9TTC1_3mutA
GGSEAAKGGG	AVIRE_P03360_3mutA
GGGSEAAKGGGGS	MLVMS_P03355_PLV919
GSSGGGEAAK	KORV_Q9TTC1-Pro_3mutA
EAAKGGGPAP	WMSV_P03359_3mut
GSSPAP	XMRV6_A1Z651_3mutA
AEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	SFV3L_P27401-Pro
GGSEAAKGSS	MLVMS_P03355_PLV919
GSSGGSEAAK	KORV_Q9TTC1-Pro_3mutA
GGSEAAKGSS	KORV_Q9TTC1-Pro_3mutA
EAAKGGG	AVIRE_P03360_3mutA
GSSGGSEAAK	BAEVM_P10272_3mutA
GGGSEAAKGGGGS	KORV_Q9TTC1-Pro_3mut
PAPGSSEAAK	MLVMS_P03355_3mut
PAPEAAK	WMSV_P03359_3mut
PAPGGSGSS	PERV_Q4VFZ2_3mutA_WS
PAPGSS	BAEVM_P10272_3mut
PAPGGGGGS	MLVMS_P03355_3mut
EAAKPAPGSS	MLVBM_Q7SVK7_3mutA_WS
GSSPAPGGS	MLVMS_P03355_PLV919
GGSGSSEAAK	MLVMS_P03355_3mut
GGGGG	KORV_Q9TTC1-Pro_3mutA
EAAKEAAKEAAKEAAK	MLVBM_Q7SVK7_3mut
GGSPAPGSS	MLVMS_P03355_PLV919
PAPAPAPAPAP	MLVCB_P08361_3mut
GGSGSSPAP	WMSV_P03359_3mutA
EAAKGGSGGG	PERV_Q4VFZ2_3mutA_WS
GSSGSSGSSGSSGSS	PERV_Q4VFZ2_3mut
AEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	KORV_Q9TTC1_3mutA
GSSGGGEAAK	WMSV_P03359_3mutA

Linker amino acid sequence	RT domain name
GSSGGSEAAK	FLV_P10273_3mutA
GGGGGGGG	PERV_Q4VFZ2_3mut
PAPGGSEAAK	FLV_P10273_3mutA
GGGGSSPAP	BAEVM_P10272_3mutA
PAPAPAPAP	WMSV_P03359_3mut
GGSEAAKPAP	PERV_Q4VFZ2_3mut
PAPGGSGGG	BAEVM_P10272_3mutA
EAAKEAAKEAAKEAAKEAAKEAAK	MLVMS_P03355_3mut
GGGGSGGGSGGGGS	PERV_Q4VFZ2_3mut
GGSGGGPAP	PERV_Q4VFZ2_3mut
GGGPAPEAAK	MLVFF_P26809_3mut
GGGGSGSS	MLVMS_P03355_3mutA_WS
GSS	MLVCB_P08361_3mut
GGGGSPAP	MLVMS_P03355_PLV919
GGSPAP	MLVAV_P03356_3mutA
GGGPAPGGS	KORV_Q9TTC1-Pro_3mutA
PAPGGSGGG	FLV_P10273_3mutA
PAPGGSGGG	WMSV_P03359_3mutA
PAPGGS	MLVBM_Q7SVK7_3mutA_WS
GGGEAAKGSS	PERV_Q4VFZ2_3mutA_WS
GGSEAAKGSS	MLVBM_Q7SVK7_3mutA_WS
PAPGGSEAAK	MLVCB_P08361_3mut
GGSEAAKGGG	XMRV6_A1Z651_3mutA
GGSGGGGSS	WMSV_P03359_3mut
GGGEAAKPAP	KORV_Q9TTC1_3mutA
EAAKGSS	KORV_Q9TTC1-Pro_3mut
PAPEAAKGSS	MLVFF_P26809_3mut
GSAGSAAGSGEF	PERV_Q4VFZ2_3mut
EAAKGSGGGS	WMSV_P03359_3mut
EAAKGSSPAP	WMSV_P03359_3mutA
GGGGSEAAKGSGGS	XMRV6_A1Z651_3mutA
GSSEAAKPAP	SFV3L_P27401-Pro_2mutA
GGGGGG	PERV_Q4VFZ2_3mutA_WS
PAPGGS	BAEVM_P10272_3mut
PAP	AVIRE_P03360_3mut
PAPAPAP	MLVBM_Q7SVK7_3mutA_WS
GGGG	PERV_Q4VFZ2_3mutA_WS
GSSGGSEAAK	MLVBM_Q7SVK7_3mut
GGSGGGGSS	MLVFF_P26809_3mut
GGGGSSGGS	AVIRE_P03360_3mutA

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Linker amino acid sequence	RT domain name
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PAPGGGGSS	PERV_Q4VFZ2_3mut
GSSGSSGSS	WMSV_P03359_3mut
EAAAKEAAKEAAKEAAK	WMSV_P03359_3mut
GGS	AVIRE_P03360_3mut
EAAKPAPGSS	MLVFF_P26809_3mut
EAAKGGG	KORV_Q9TTC1_3mut
PAPGSSEAAK	MLVMS_P03355_3mut
PAPGSSGSS	MLVMS_P03355_PLV919
GSSPAPEAAK	MLVMS_P03355_3mut
GSSGSSGSSGSSGSSGSS	WMSV_P03359_3mutA
GGGGS	BAEVM_P10272_3mut
GSSPAP	MLVMS_P03355_3mut
EAAKGGGGSEAAK	KORV_Q9TTC1-Pro_3mutA
EAAKEAAK	WMSV_P03359_3mutA
GGGGSSGSS	MLVCB_P08361_3mutA
PAPGGSEAAK	BAEVM_P10272_3mut
EAAKGGSPAP	MLVFF_P26809_3mut
GSSGGSGGG	MLVBM_Q7SVK7_3mutA_WS
GSSGGS	PERV_Q4VFZ2_3mut
PAPGGSGSS	PERV_Q4VFZ2_3mutA_WS
EAAKGGSGSS	KORV_Q9TTC1-Pro_3mutA
PAPAP	MLVCB_P08361_3mut
EAAKGGSPAP	PERV_Q4VFZ2_3mutA_WS
EAAKPAPGGG	MLVMS_P03355_PLV919
GGGGSGGGSGGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mut
EAAKGGGSS	MLVMS_P03355_PLV919
PAPEAAK	PERV_Q4VFZ2_3mut
EAAKPAPGSS	BAEVM_P10272_3mutA
GGSPAP	PERV_Q4VFZ2_3mutA_WS
GGSGGS	BAEVM_P10272_3mutA
PAPEAAKGSS	KORV_Q9TTC1_3mut
PAPGSS	MLVMS_P03355_PLV919
PAPAPAPAPAP	MLVAV_P03356_3mutA
GGG	XMRV6_A1Z651_3mutA
GGGPAP	PERV_Q4VFZ2_3mutA_WS
GSSPAPEAAK	KORV_Q9TTC1_3mutA
PAP	BAEVM_P10272_3mutA
GGSPAP	BAEVM_P10272_3mutA
PAPEAAKGSS	MLVMS_P03355_PLV919

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Linker amino acid sequence	RT domain name
PAPAPAPAPAPAP	KORV_Q9TTC1_3mutA
GGGGSS	BAEVM_P10272_3mut
GGGGSEAAKGGGGS	AVIRE_P03360_3mut
GSSPAPEAAK	KORV_Q9TTC1-Pro_3mutA
PAPEAAKGGG	MLVBM_Q7SVK7_3mut
EAAAKEAAK	WMSV_P03359_3mut
EAAK	SFV3L_P27401-Pro_2mutA
GSSGGSGGG	XMRV6_A1Z651_3mutA
GGGEAAKPAP	WMSV_P03359_3mutA
GGSGGS	MLVFF_P26809_3mutA
EAAAKEAAKEAAKEAAKEAAKEAAK	FOAMV_P14350_2mutA
GGGGG	MLVAV_P03356_3mutA
GSSGGSEAAK	BAEVM_P10272_3mut
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	SFV1_P23074
GGSGGGPAP	MLVCB_P08361_3mut
GGSGGS	PERV_Q4VFZ2_3mut
SGSETPGTSESATPES	MLVFF_P26809_3mut
EAAKGGSPAP	MLVMS_P03355_3mut
PAPAP	PERV_Q4VFZ2_3mut
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	MLVBM_Q7SVK7_3mut
GGGGGS	BAEVM_P10272_3mutA
EAAAKEAAK	AVIRE_P03360_3mut
GSSGGSEAAK	PERV_Q4VFZ2_3mut
GGGEAAK	WMSV_P03359_3mut
GSSGGGEAAK	AVIRE_P03360_3mutA
GGG	XMRV6_A1Z651_3mut
GGGGSEAAKGGGGS	BAEVM_P10272_3mut
GGGG	MLVMS_P03355_3mut
GGSGGS	MLVMS_P03355_3mutA_WS
GGSGGGGS	MLVBM_Q7SVK7_3mutA_WS
GSSPAPGGS	PERV_Q4VFZ2_3mut
GSSPAPEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKGGGS	WMSV_P03359_3mut
GGSGSGSGSGGS	PERV_Q4VFZ2_3mut
GGGGSSEAAK	KORV_Q9TTC1-Pro_3mut
PAPAPAPAPAPAP	MLVAV_P03356_3mut
EAAKGSSGGG	MLVMS_P03355_PLV919
GGGGG	MLVBM_Q7SVK7_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	FFV_O93209_2mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	KORV_Q9TTC1-Pro_3mut

Linker amino acid sequence	RT domain name
GGSPAPGGG	MLVMS_P03355_3mutA_WS
GGGEAAKGGG	MLVMS_P03355_3mut
GGGEAAK	PERV_Q4VFZ2_3mut
PAPEAAKGGG	MLVMS_P03355_3mut
GSSGSSGSSGSSGSSGSS	BAEVM_P10272_3mutA
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	GALV_P21414_3mutA
EAAKGGSPAP	FFV_O93209-Pro
EAAKEAAK	MLVFF_P26809_3mut
GGGGSGGGSGGGSGGGSGGGSGGGG	PERV_Q4VFZ2_3mutA_WS
GGSGGSGGGSGG	MLVAV_P03356_3mutA
EAAKEAAKEAAKEAAKEAAK	SFV3L_P27401_2mutA
GSSGSSGSSGSSGSSGSS	BAEVM_P10272_3mut
GGGGG	MLVMS_P03355_PLV919
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	SFV1_P23074
GGGGSGGGG	KORV_Q9TTC1-Pro_3mutA
GGGGSGGGG	MLVMS_P03355_3mut
GGSGSS	KORV_Q9TTC1_3mutA
GSSPAPGGG	PERV_Q4VFZ2_3mut
GSSGGSPAP	PERV_Q4VFZ2_3mutA_WS
PAPGGG	PERV_Q4VFZ2_3mutA_WS
GGSPAPEAAK	FOAMV_P14350_2mutA
GGGPAPGGG	SFV3L_P27401_2mut
PAPGSSGGG	MLVCB_P08361_3mut
GSSGGGEAAK	AVIRE_P03360_3mut
GSSGGG	XMRV6_A12651_3mut
GSSGSS	PERV_Q4VFZ2_3mut
GSSGGG	MLVAV_P03356_3mutA
PAPGGGGG	PERV_Q4VFZ2_3mut
GSSEAAK	MLVMS_P03355_3mut
PAPGGG	FLV_P10273_3mutA
GGGGSGGGG	PERV_Q4VFZ2_3mut
GSSGSS	MLVMS_P03355_PLV919
GGGGSGGGG	SFV3L_P27401_2mut
EAAKGGSGSS	FLV_P10273_3mutA
GSSEAAKGGG	MLVMS_P03355_3mutA_WS
PAPGSSEAAK	SFV3L_P27401_2mutA
GGGGSGGGG	SFV3L_P27401-Pro_2mutA
PAPGSSEAAK	PERV_Q4VFZ2_3mut
PAPGSSEAAK	PERV_Q4VFZ2
GGSPAPGGG	AVIRE_P03360_3mut

Linker amino acid sequence	RT domain name
GGGGGS	PERV_Q4VFZ2_3mutA_WS
GGGGSSGGGS	PERV_Q4VFZ2_3mut
PAPAPAPAP	AVIRE_P03360_3mutA
GGSGGS	WMSV_P03359_3mutA
GGGPAPGGS	PERV_Q4VFZ2_3mut
GGSGSGSGSGSGGS	MLVMS_P03355_PLV919
GGSGGG	PERV_Q4VFZ2_3mut
EAAAKEAAK	SFV3L_P27401_2mut
PAPGSS	XMRV6_A1Z651_3mut
GSSEAAK	MLVFF_P26809_3mut
GGSPAPGGG	MLVMS_P03355_3mut
EAAAKGGG	WMSV_P03359_3mutA
GSSEAAKGGGS	PERV_Q4VFZ2_3mutA_WS
GSSGGSPAP	FFV_O93209
GGGGGS	KORV_Q9TTC1-Pro_3mut
GSSGGG	MLVCB_P08361_3mut
GSSGSS	MLVCB_P08361_3mutA
GGSEAAKPAP	BAEVM_P10272_3mut
EAAAKGGGGSS	MLVCB_P08361_3mut
EAAAKPAPGGS	KORV_Q9TTC1-Pro_3mutA
GSSGSSGSSGSSGSS	MLVAV_P03356_3mutA
GGGGSEAAKGGGGGS	PERV_Q4VFZ2_3mutA_WS
GSSGSS	KORV_Q9TTC1-Pro_3mut
GSS	SFV3L_P27401-Pro_2mutA
PAPAP	BAEVM_P10272_3mut
EAAKPAP	BAEVM_P10272
EAAAKEAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mut
GGGGGGG	PERV_Q4VFZ2_3mutA_WS
GGGGS	MLVMS_P03355_3mut
GSSGGG	FLV_P10273_3mutA
PAPAPAPAPAP	FLV_P10273_3mut
EAAKEAAKEAAK	WMSV_P03359_3mutA
GSSGGS	MLVBM_Q7SVK7_3mutA_WS
EAAKPAPGGG	MLVMS_P03355_3mut
GSSPAPGGS	WMSV_P03359_3mut
PAPGSSGGG	PERV_Q4VFZ2_3mutA_WS
GSSGGG	AVIRE_P03360_3mutA
PAPGSGSS	MLVFF_P26809_3mut
PAPGSS	PERV_Q4VFZ2_3mut
GGGGSGSS	WMSV_P03359_3mutA

Linker amino acid sequence	RT domain name
EAAAKGGGGSS	MLVBM_Q7SVK7_3mutA_WS
GGGGGGG	BAEVM_P10272_3mut
PAPEAAKGSS	MLVMS_P03355_3mut
GGSGGGEAAK	MLVMS_P03355_PLV919
EAAAKGGGGGS	MLVCB_P08361_3mut
PAPGGS	KORV_Q9TTC1-Pro_3mut
GGGG	FLV_P10273_3mutA
EAAAKGGSGSS	MLVBM_Q7SVK7_3mutA_WS
GGGGSSGGG	MLVMS_P03355_3mutA_WS
GGGGGGGG	WMSV_P03359_3mut
GGSGSSGGG	MLVMS_P03355_PLV919
GSSEAAKGSS	KORV_Q9TTC1-Pro_3mutA
EAAKPAPGSS	MLVCB_P08361_3mut
GGSPAPGSS	KORV_Q9TTC1_3mutA
PAPGSSGGG	BAEVM_P10272_3mut
EAAKPAPGSS	WMSV_P03359_3mut
GGSPAPEAAK	XMRV6_A1Z651_3mutA
GSSPAP	FLV_P10273_3mutA
GSS	BAEVM_P10272_3mutA
EAAKPAPGSS	FLV_P10273_3mutA
GGSGSPAP	FLV_P10273_3mutA
PAPGSSGGG	MLVMS_P03355_3mut
GSAGSAAGSGEF	PERV_Q4VFZ2_3mutA_WS
GSSGGSEAAK	KORV_Q9TTC1_3mutA
GSSGGG	MLVMS_P03355_3mutA_WS
EAAAKGGGGSEAAK	SFV3L_P27401_2mut
GSSGGG	PERV_Q4VFZ2_3mutA_WS
GGSPAPEAAK	FLV_P10273_3mut
GGSEAAKGSS	PERV_Q4VFZ2_3mutA_WS
GSSPAPEAAK	PERV_Q4VFZ2_3mutA_WS
GGSGSSGGG	PERV_Q4VFZ2_3mut
GGGG	AVIRE_P03360_3mutA
GGSEAAKPAP	WMSV_P03359_3mut
GSSGGSPAP	MLVAV_P03356_3mutA
GSSGGSEAAK	MLVMS_P03355_3mut
PAPEAAKGSS	KORV_Q9TTC1-Pro_3mut
GGSPAP	PERV_Q4VFZ2_3mutA_WS
GGSEAAK	MLVAV_P03356_3mutA
EAAAKGGGGSEAAK	KORV_Q9TTC1-Pro_3mut
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVMS_P03355_PLV919

Linker amino acid sequence	RT domain name
GSSEAAAK	KORV_Q9TTC1_3mutA
GGG	AVIRE_P03360
GGSEAAAKGSS	MLVBM_Q7SVK7_3mut
GGSEAAAKGSS	MLVMS_P03355_3mut
GGSPAEEAAK	MLVCB_P08361_3mut
GGSGGGEAAK	MLVCB_P08361_3mut
GGSEAAKPAP	MLVMS_P03355_3mutA_WS
EAAAKGGSGSS	KORV_Q9TTC1-Pro_3mut
GGGEAAAKGGS	MLVCB_P08361_3mut
EAAAKGGGSEAAK	FLV_P10273_3mutA
GGSPAP	MLVFF_P26809_3mut
GGGGSSGGS	XMRV6_A1Z651_3mutA
PAP	MLVCB_P08361_3mut
GGS	SFV3L_P27401-Pro_2mutA
GGGGSGGGGS	MLVMS_P03355_3mut
GGGEAAAKGGS	MLVAV_P03356_3mutA
GSSGSSGSSGSSGSSGSS	MLVMS_P03355_PLV919
PAPGSS	MLVCB_P08361_3mut
GGSGGSGGS	MLVMS_P03355_PLV919
PAPGGSGGG	FLV_P10273_3mutA
GGGGSGGGSGGGSGGS	FLV_P10273_3mut
GGSGSSGGG	KORV_Q9TTC1-Pro_3mutA
GGSGGSGGS	GALV_P21414_3mutA
GGGEAAAKGGS	WMSV_P03359_3mut
SGSETPGTSESATPES	KORV_Q9TTC1_3mutA
EAAAKGGGGGS	KORV_Q9TTC1-Pro_3mut
EAAAKGSSPAP	BAEVM_P10272_3mut
GGGG	MLVCB_P08361_3mut
GGGGSGGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mut
GSSGSGGG	MLVMS_P03355_PLV919
GGSGSS	MLVFF_P26809_3mut
EAAAKGGS	AVIRE_P03360_3mutA
GSSEAAAKGGS	MLVBM_Q7SVK7_3mutA_WS
EAAAKPAPGGG	WMSV_P03359_3mut
PAPGSSGGG	MLVCB_P08361_3mutA
GGGGSSSEAAK	KORV_Q9TTC1-Pro_3mutA
GSSEAAKPAP	BAEVM_P10272_3mutA
PAPGGGEAAK	MLVBM_Q7SVK7_3mutA_WS
GGSGGGEAAK	MLVCB_P08361_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGGS	FFV_O93209

Linker amino acid sequence	RT domain name
EAAAKGGGGGS	GALV_P21414_3mutA
GGSPAPGGG	MLVMS_P03355_3mut
GSSGSSGSS	FLV_P10273_3mutA
EAAAK	MLVBM_Q7SVK7_3mut
GGGGSSGSS	MLVMS_P03355_3mut
GGSGSSPAP	PERV_Q4VFZ2_3mut
EAAAKEAAKEAAKEAAK	BAEVM_P10272_3mut
GGGPAPGSS	MLVMS_P03355_3mut
GSSPAPGSS	PERV_Q4VFZ2_3mutA_WS
PAPAP	FLV_P10273_3mutA
PAPAPAPAP	PERV_Q4VFZ2_3mut
GGGGGSEAAK	GALV_P21414_3mutA
GGGGGSGSS	BAEVM_P10272_3mutA
GGGEAAKGSS	KORV_Q9TTC1_3mutA
GGGGGSPAP	AVIRE_P03360_3mut
GGGGGSEAAK	SFV3L_P27401_2mutA
GSS	KORV_Q9TTC1_3mutA
GGGGGGG	PERV_Q4VFZ2_3mut
SGSETPGTSESATPES	SFV3L_P27401_2mutA
EAAAKGSGGG	MLVMS_P03355_3mut
GGGGS	MLVFF_P26809_3mut
EAAAKGSSGGG	BAEVM_P10272_3mut
EAAKPAPGSS	MLVF5_P26810_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	SFV3L_P27401_2mutA
GGSPAPGGG	WMSV_P03359_3mutA
GSAGSAAGSGEF	MLVFF_P26809_3mut
GGGGSSGSS	MLVMS_P03355_3mutA_WS
GGGGGGG	MLVCB_P08361_3mut
GSSEAAK	WMSV_P03359_3mut
PAPGSS	FLV_P10273_3mutA
GSSGGG	PERV_Q4VFZ2_3mutA_WS
PAPGGG	MLVFF_P26809_3mut
GGGGGSPAP	MLVMS_P03355_3mut
GGSEAAK	XMRV6_A1Z651_3mut
GSSGGG	PERV_Q4VFZ2_3mut
GGSGGGSGSGGS	MLVMS_P03355_3mut
PAPAP	AVIRE_P03360_3mut
GGSEAAK	PERV_Q4VFZ2_3mut
GGGGS	MLVMS_P03355_PLV919
GGGG	BAEVM_P10272_3mutA

Linker amino acid sequence	RT domain name
EAAAKGGGGSS	MLVCB_P08361_3mutA
EAAAKEAAKEAAK	GALV_P21414_3mutA
PAPGGGEAAK	KORV_Q9TTC1
EAAAKGGSPAP	MLVMS_P03355_3mut
GGSGSSEAAK	MLVMS_P03355_3mut
GGSPAPEAAK	FLV_P10273_3mutA
GGGGGGG	PERV_Q4VFZ2_3mut
EAAKEAAKEAAKEAAKEAAKEAAK	SFV1_P23074_2mutA
EAAAKGSSGGS	MLVMS_P03355_3mut
GSSEAAKPAP	MLVFF_P26809_3mut
GGGGSS	FLV_P10273_3mutA
EAAAKGGSGGG	AVIRE_P03360_3mutA
GGSGGS	PERV_Q4VFZ2_3mutA_WS
GGGGGGSPAP	AVIRE_P03360_3mutA
EAAKEAAKEAAK	XMRV6_A1Z651_3mut
PAPEAAKGGS	FLV_P10273_3mutA
GSSGGSEAAK	MLVCB_P08361_3mut
EAAAKGGSGGG	MLVMS_P03355
GGSGGGPAP	MLVMS_P03355_3mut
GGS	XMRV6_A1Z651_3mut
GGSEAAKPAP	MLVFF_P26809_3mut
EAAAKGGG	MLVMS_P03355_PLV919
GSSGSSGSSGSS	WMSV_P03359_3mut
GGSGSSPAP	PERV_Q4VFZ2_3mut
GGGEAAK	MLVMS_P03355_3mutA_WS
GSSPAPGGS	KORV_Q9TTC1-Pro_3mutA
GSSEAAKGGG	SFV3L_P27401_2mut
EAAKPAPGGS	MLVCB_P08361_3mut
GGSGGGEAAK	PERV_Q4VFZ2
GGSGSS	MLVCB_P08361_3mut
GGSGGGEAAK	MLVBM_Q7SVK7_3mutA_WS
GGSGSGSGSGSGSGGS	FLV_P10273_3mut
PAPEAAKGGS	MLVMS_P03355_3mut
EAAAKGSSGGS	WMSV_P03359_3mutA
GGSGSSEAAK	MLVCB_P08361_3mut
GGSGSSEAAK	KORV_Q9TTC1_3mutA
GSSGGSGGG	MLVMS_P03355_PLV919
EAAAKGGSGGG	SFV3L_P27401-Pro_2mutA
GGSGGS	AVIRE_P03360_3mutA
GSAGSAAGSGEF	MLVMS_P03355_PLV919

Linker amino acid sequence	RT domain name
GGSGSS	GALV_P21414_3mutA
GGGG	MLVFF_P26809_3mutA
GGGGSGGGSGGGSGGGGS	WMSV_P03359_3mut
SGSETPGTSESATPES	BAEVM_P10272_3mut
EAAAKEAAKEAAKEAAK	FOAMV_P14350_2mutA
GGGEAAKGGS	FLV_P10273_3mutA
GSSGGEAAK	MLVFF_P26809_3mut
EAAKGGGGSS	MLVAV_P03356_3mut
PAPGGEAAK	KORV_Q9TTC1-Pro_3mut
EAAK	XMRV6_A1Z651_3mut
GSSGSSGSSGSSGSSGSS	PERV_Q4VFZ2_3mut
GGGG	MLVCB_P08361_3mutA
GSSGSS	WMSV_P03359_3mutA
GSSGGSPAP	AVIRE_P03360_3mut
GSSGGSGGS	MLVCB_P08361_3mut
EAAKGGGPAP	FLV_P10273_3mutA
GGGGSGGGGS	MLVCB_P08361_3mut
GGSEAAKGSS	PERV_Q4VFZ2_3mutA_WS
EAAKEAAKEAAKEAAKEAAKEAAK	SFV3L_P27401_2mutA
GSSGSSEAAK	PERV_Q4VFZ2_3mutA_WS
EAAKEAAKEAAKEAAK	SFV3L_P27401-Pro_2mutA
GSSEAAKGGS	FLV_P10273_3mutA
GGSGSS	PERV_Q4VFZ2
GGSGSSEAAK	SFV3L_P27401-Pro_2mutA
GSSGSSGSS	XMRV6_A1Z651_3mutA
EAAKGSSPAP	KORV_Q9TTC1_3mutA
EAAKPAP	FLV_P10273_3mutA
GGSGSSEAAK	KORV_Q9TTC1-Pro_3mut
GGGGSGGGSGGGSGGGSGGGSGGGGS	KORV_Q9TTC1_3mutA
GGGGSGGGSGGGGS	KORV_Q9TTC1-Pro_3mutA
GGGGGGG	FLV_P10273_3mut
EAAKGSS	WMSV_P03359_3mut
EAAKGGGPAP	MLVCB_P08361_3mut
GSSGSS	MLVBM_Q7SVK7_3mutA_WS
EAAKGGGGGS	MLVFF_P26809_3mut
GGSGGEAAK	FLV_P10273_3mutA
PAPGSS	MLVFF_P26809_3mutA
PAPGSS	BAEVM_P10272_3mutA
GGSPAPGSS	AVIRE_P03360_3mut
GGGSSEAAK	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
GSSGGGGGS	FFV_O93209-Pro
EAAAKGSSPAP	PERV_Q4VFZ2_3mut
GSSPAPGGS	PERV_Q4VFZ2_3mut
GGGGGG	BAEVM_P10272_3mut
EAAAKGGGGSS	PERV_Q4VFZ2_3mutA_WS
PAPGSEAAK	KORV_Q9TTC1_3mutA
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVMS_P03355_3mutA_WS
GSSGGSSGGSS	MLVMS_P03355_3mut
EAAAKGSSGGG	MLVMS_P03355_PLV919
GGSEAAKPAP	AVIRE_P03360_3mutA
GSSGGSSGGSSGGSS	WMSV_P03359_3mutA
GGGEAAKPAP	FLV_P10273_3mutA
PAPGSSGGG	KORV_Q9TTC1_3mutA
GSSGSS	MLVMS_P03355_3mutA_WS
PAPEAAK	BAEVM_P10272_3mut
GGGPAPGSS	PERV_Q4VFZ2
GSSGGSPAP	MLVFF_P26809_3mut
GGGGSS	SFV3L_P27401_2mut
PAPEAAKGSS	SFV3L_P27401_2mut
GGSGGGPAP	XMRV6_A1Z651_3mutA
PAPGGS	BAEVM_P10272_3mutA
EAAAKGGGGGS	AVIRE_P03360_3mut
GSSGGSPAP	KORV_Q9TTC1-Pro_3mutA
GSSGGGGGS	WMSV_P03359_3mut
GGGEAAKGGS	AVIRE_P03360_3mut
GGGEAAKGSS	BAEVM_P10272_3mut
PAPEAAKGSS	MLVAV_P03356_3mutA
GSSGGSSGGSSGGSS	MLVCB_P08361_3mut
GGSPAPGSS	FLV_P10273_3mutA
EAAAKGSSPAP	BAEVM_P10272_3mutA
GGSGGGGGSGSGSGGS	PERV_Q4VFZ2
GGGGSSEAAK	FLV_P10273_3mutA
GGGGSSPAP	FFV_O93209
GSSGGSPAP	MLVMS_P03355_3mut
GGGPAPGSS	MLVMS_P03355_PLV919
PAPGSSGGS	PERV_Q4VFZ2_3mut
GGGGSPAP	MLVFF_P26809_3mut
SGSETPGTSESATPES	MLVMS_P03355_3mutA_WS
GSSGGSSGGSSGGSS	KORV_Q9TTC1_3mutA
GSSPAPGGG	WMSV_P03359_3mut

Linker amino acid sequence	RT domain name
PAPAPAPAPAPAP	SFV3L_P27401_2mutA
GGGPAPGGS	MLVMS_P03355_3mut
PAPGGSEAAK	WMSV_P03359_3mut
GGGGSSEAAK	FFV_093209-Pro
GGSPAPGGG	FLV_P10273_3mutA
GSSPAPEAAK	AVIRE_P03360_3mut
GGGEAAK	FLV_P10273_3mutA
PAPEAAKGGG	MLVCB_P08361_3mut
GGSPAPGGG	MLVCB_P08361_3mut
GGSGGGGSS	BAEVM_P10272_3mutA
GSSPAPEAAK	MLVCB_P08361_3mut
GGSPAPGGG	KORV_Q9TTC1-Pro_3mutA
PAPGGSGSS	KORV_Q9TTC1_3mutA
GSSPAP	KORV_Q9TTC1-Pro_3mutA
SGSETPGTSESATPES	MLVMS_P03355
GSSGSSGSS	MLVAV_P03356_3mutA
PAPGSSGGS	PERV_Q4VFZ2_3mutA_WS
PAPGGS	KORV_Q9TTC1-Pro_3mutA
PAPEAAKGGG	SFV3L_P27401-Pro_2mutA
GGSGSGGGS	BAEVM_P10272_3mut
PAPGGS	MLVFF_P26809_3mut
GSSGGSPAP	MLVMS_P03355_PLV919
GSSGGGGGS	FLV_P10273_3mutA
GGGGGSPAP	KORV_Q9TTC1-Pro_3mut
EAAKAPAGSS	SFV3L_P27401-Pro_2mutA
EAAKGGSPAP	KORV_Q9TTC1-Pro
GGGPAPEAAK	MLVMS_P03355_PLV919
GGSEAAKGSS	MLVMS_P03355
PAPEAAKGSS	KORV_Q9TTC1_3mutA
PAPEAAKGGG	WMSV_P03359_3mutA
GSSGGG	PERV_Q4VFZ2_3mutA_WS
EAAKGGGGSS	MLVMS_P03355_PLV919
EAAKGGSPAP	AVIRE_P03360_3mutA
GGGGSSGGS	MLVMS_P03355_PLV919
PAPEAAKGSS	PERV_Q4VFZ2_3mutA_WS
EAAKGGGGGS	BAEVM_P10272_3mut
GSSGGGGGS	MLVMS_P03355_3mut
PAPAPAPAP	KORV_Q9TTC1_3mutA
GGSGSGSGSGGS	MLVAV_P03356_3mut
PAPAPAPAP	SFV3L_P27401_2mut

Linker amino acid sequence	RT domain name
GSSSEAAKPAP	MLVMS_P03355_3mut
GGSGGGEAAK	SFV3L_P27401_2mutA
GSSGGSGGG	MLVMS_P03355_3mutA_WS
GGGGGSPAP	MLVCB_P08361_3mutA
GGGEAAKGSS	XMRV6_A12651_3mutA
GGGGSSPAP	BAEVM_P10272_3mut
GGSGGG	PERV_Q4VFZ2_3mut
GGGGSS	MLVBM_Q7SVK7_3mutA_WS
EAAAKGSSGGS	PERV_Q4VFZ2_3mutA_WS
GSSGGGGGS	PERV_Q4VFZ2
EAAAKGSSGGS	PERV_Q4VFZ2_3mut
EAAAKEAAK	MLVAV_P03356_3mut
GSSGGGEAAK	MLVAV_P03356_3mut
GSSPAPGGG	XMRV6_A12651_3mut
GGGGSGGGSGGGGS	PERV_Q4VFZ2_3mut
EAAAKEAAKEAAKEAAK	KORV_Q9TTC1_3mutA
EAAAKGGSGSS	MLVBM_Q7SVK7_3mut
PAPEAAK	BLVJ_P03361
GSSGGG	FFV_O93209-Pro
GGSGGGEAAK	KORV_Q9TTC1-Pro_3mutA
EAAK	FLV_P10273_3mutA
GGGGSSPAP	MLVMS_P03355_3mut
GSS	SFV3L_P27401-Pro_2mut
PAPEAAKGSS	BAEVM_P10272_3mut
GGGGGSPAP	PERV_Q4VFZ2_3mut
GSSGSSGSS	BAEVM_P10272_3mutA
GGGGSGGGSGGGSGGGGS	SFV1_P23074_2mut
GGGGSEAAK	SFV3L_P27401_2mutA
GGGGSGGGSGGGSGGGGS	FOAMV_P14350-Pro_2mut
PAPGSSEAAK	MLVBM_Q7SVK7_3mutA_WS
GGGGSGGSS	MLVFF_P26809_3mutA
GGSEAAKGGG	MLVBM_Q7SVK7_3mut
PAPGSSGGG	PERV_Q4VFZ2
GSS	PERV_Q4VFZ2_3mutA_WS
EAAAKGGSGSS	FLV_P10273_3mut
GGGEAAK	WMSV_P03359_3mutA
GGSEAAKPAP	MLVBM_Q7SVK7_3mut
SGSETPGTSESATPES	FOAMV_P14350-Pro_2mutA
EAAAKPAPGSS	AVIRE_P03360_3mut
EAAAKGGGGGS	KORV_Q9TTC1-Pro_3mutA

Linker amino acid sequence	RT domain name
GGGGS	PERV_Q4VFZ2_3mut
GGSEAAAKGSS	MLVFF_P26809_3mutA
GGSEAAAKGGG	AVIRE_P03360
GGSGSGSGSGSGSGSGGS	SFV3L_P27401_2mut
GGSEAAAKGSS	SFV3L_P27401-Pro_2mutA
GGGEAAAKPAP	MLVCB_P08361_3mut
GGSEAAAK	MLVMS_P03355_PLV919
GGSPAPGSS	KORV_Q9TTC1-Pro_3mutA
GSSPAPEAAK	WMSV_P03359_3mutA
GGSGSS	KORV_Q9TTC1-Pro_3mutA
PAPGGGGGS	AVIRE_P03360_3mut
PAPEAAAKGSS	FFV_O93209-Pro
GGSGGGEAAK	WMSV_P03359_3mut
PAPGGG	MLVMS_P03355_3mut
EAAAKGGG	FLV_P10273_3mutA
GSSGSSGSSGSS	MLVCB_P08361_3mut
EAAAKGGSGGG	FFV_O93209
GSSPAPGSS	PERV_Q4VFZ2_3mutA_WS
GSSPAPGSS	MLVCB_P08361_3mut
GGGPAP	WMSV_P03359_3mutA
GGGPAP	KORV_Q9TTC1_3mutA
GGSPAPGSS	KORV_Q9TTC1-Pro_3mut
PAPAP	MLVMS_P03355_3mut
GGGGGGG	MLVMS_P03355_3mut
GGGGG	KORV_Q9TTC1-Pro_3mut
GSAGSAAGSGEF	FOAMV_P14350_2mutA
PAPAP	KORV_Q9TTC1-Pro_3mutA
GGSEAAAKGGG	SFV3L_P27401-Pro_2mutA
PAPAP	WMSV_P03359_3mut
GGGSGSGSGSGSGGS	SFV3L_P27401_2mut
PAPGGS	KORV_Q9TTC1_3mutA
GGGEAAAKPAP	FLV_P10273_3mut
GGGGGS	MLVAV_P03356_3mutA
GSSEAAAKGGG	WMSV_P03359_3mut
EAAAKGGGGSS	GALV_P21414_3mutA
GSSGGS	MLVAV_P03356_3mutA
GSSGGG	MLVBM_Q7SVK7_3mut
PAPAPAP	SFV3L_P27401-Pro_2mutA
GGGG	KORV_Q9TTC1_3mutA
EAAAKPAPGGS	MLVFF_P26809_3mut

Linker amino acid sequence	RT domain name
GGGGSGGGGS	XMRV6_A1Z651_3mut
EAAAKGGG	MLVCB_P08361_3mut
GGGGSSPAP	KORV_Q9TTC1_3mutA
GSSEAAKGGG	KORV_Q9TTC1-Pro_3mutA
GGGGG	BLVJ_P03361_2mutB
GGGEAAKGSS	FFV_O93209-Pro
GSSGSSGSS	BAEVM_P10272_3mut
GSSGGSPAP	PERV_Q4VFZ2_3mut
EAAAKGSS	KORV_Q9TTC1_3mut
GGSPAPEAAK	AVIRE_P03360_3mut
GGSEAAK	WMSV_P03359_3mut
GSSGGS	KORV_Q9TTC1-Pro_3mutA
GGGPAPEAAK	KORV_Q9TTC1_3mutA
PAPGSS	WMSV_P03359_3mutA
GGSEAAKGSS	FLV_P10273_3mutA
EAAAKEAAKEAAKEAAKEAAK	SFV3L_P27401
GSSEAAKGGG	SFV3L_P27401-Pro_2mutA
GGGGSEAAKGGGGS	KORV_Q9TTC1-Pro_3mutA
GGSGGSGGS	WMSV_P03359_3mut
GGGGSGGSS	KORV_Q9TTC1-Pro
GGGGSGGGSGGGSGGGGS	MLVMS_P03355_3mut
EAAAKGGG	PERV_Q4VFZ2
GGSEAAKGGG	KORV_Q9TTC1-Pro_3mut
GSSGSGGG	PERV_Q4VFZ2_3mutA_WS
GGGGGS	PERV_Q4VFZ2_3mut
GSAGSAAGSGEF	PERV_Q4VFZ2
PAPEAAKGGSS	BAEVM_P10272_3mutA
GSSPAPGGG	MLVCB_P08361_3mut
GGGGSSPAP	KORV_Q9TTC1-Pro_3mutA
PAPGSGGG	MLVFF_P26809_3mut
GSSPAP	KORV_Q9TTC1_3mutA
PAPGSS	SFV3L_P27401-Pro_2mut
GGSGGGGSS	MLVMS_P03355_PLV919
GSSGGS	WMSV_P03359_3mutA
EAAAKGGGGGS	PERV_Q4VFZ2
GGGGG	KORV_Q9TTC1_3mutA
EAAAKGSS	MLVMS_P03355_PLV919
EAAAKEAAKEAAKEAAKEAAK	FLV_P10273_3mut
EAAAKEAAKEAAKEAAK	SFV3L_P27401-Pro_2mut
GSAGSAAGSGEF	SFV3L_P27401_2mutA

Linker amino acid sequence	RT domain name
GGGPAPGGS	FLV_P10273_3mutA
GGSEAAKGGG	MLVCB_P08361_3mut
PAPGGGEAAK	BAEVM_P10272_3mut
EAAAKPAPGSS	FOAMV_P14350_2mut
GGSEAAK	KORV_Q9TTC1_3mutA
GGSGSS	AVIRE_P03360
GGSPAPEAAK	MLVMS_P03355_PLV919
GGGGS	XMRV6_A12651_3mut
GGSPAPGGG	XMRV6_A12651_3mut
EAAAKPAPGGS	PERV_Q4VFZ2
GSSPAP	BAEVM_P10272_3mut
GGSGSSGGG	FLV_P10273_3mutA
PAPGGG	PERV_Q4VFZ2_3mutA_WS
GSSGGSEAAK	MLVBM_Q7SVK7_3mut
GGSEAAK	MLVMS_P03355_3mut
GGGPAPGGS	MLVFF_P26809_3mut
GSAGSAAGSGEF	MLVBM_Q7SVK7_3mutA_WS
EAAAKPAPGGS	SFVCP_Q87040
PAPGGG	PERV_Q4VFZ2_3mutA_WS
GSSPAPEAAK	MLVBM_Q7SVK7
PAPEAAK	MLVBM_Q7SVK7_3mut
PAPGGGGGS	AVIRE_P03360_3mutA
GGSEAAKPAP	MLVBM_Q7SVK7_3mut
EAAAKGSS	WMSV_P03359_3mutA
GGGEAAK	MLVFF_P26809_3mutA
EAAKEAAKEAAK	MLVMS_P03355_3mut
PAPEAAKGGG	BAEVM_P10272_3mut
PAPAPAP	MLVCB_P08361_3mut
EAAAKPAPGGS	BAEVM_P10272_3mut
GGGSGGGGS	FLV_P10273_3mut
GGGGSEAAKGGGGS	KORV_Q9TTC1_3mut
EAAK	FLV_P10273_3mut
PAPAPAP	WMSV_P03359_3mut
GGGGSEAAKGGGGS	FFV_O93209-Pro
GGSPAPEAAK	MLVMS_P03355_3mut
GGSGSSGGG	XMRV6_A12651_3mut
GGSPAPGSS	PERV_Q4VFZ2_3mut
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	SFV3L_P27401-Pro_2mutA
EAAKGGGPAP	BAEVM_P10272_3mutA
GSSGGSEAAK	MLVMS_P03355_3mutA_WS

Linker amino acid sequence	RT domain name
SGSETPGTSESATPES	PERV_Q4VFZ2_3mutA_WS
EAAAKEAAKEAAKEAAKEAAK	KORV_Q9TTC1-Pro_3mutA
GSSGSSGSS	KORV_Q9TTC1_3mutA
GSSPAPGGG	SFV3L_P27401-Pro_2mutA
GSSGGGEAAK	KORV_Q9TTC1_3mutA
GGSGGGGSS	PERV_Q4VFZ2_3mutA_WS
GSSGGGEAAK	MLVCB_P08361_3mut
GSSEAAKGGG	MLVCB_P08361_3mut
GGSGGGGSS	KORV_Q9TTC1_3mutA
GGSGSSPAP	PERV_Q4VFZ2_3mutA_WS
GSSPAP	MLVMS_P03355_3mut
GGGGSSEAAK	AVIRE_P03360
GGS	WMSV_P03359_3mut
EAAKEAAK	PERV_Q4VFZ2_3mut
PAPAPAPAP	MLVAV_P03356_3mut
GGSEAAKGGG	KORV_Q9TTC1_3mutA
PAPGGG	MLVAV_P03356_3mut
EAAKGSS	BAEVM_P10272_3mut
GGGGSGGGGS	WMSV_P03359_3mutA
GGSGSGGS	SFV3L_P27401_2mut
EAAK	MLVCB_P08361_3mut
GGGGSGGS	WMSV_P03359_3mutA
GGGPAPEAAK	MLVAV_P03356_3mutA
EAAKEAAKEAAK	FFV_O93209
GSSEAAKGGG	MLVBM_Q7SVK7_3mut
GGGPAPGGS	FLV_P10273_3mut
GGSEAAKGGG	WMSV_P03359_3mut
EAAKGSGGS	XMRV6_A12651_3mutA
EAAKGSGGG	FLV_P10273_3mutA
GGSEAAKGGG	SFV3L_P27401_2mutA
GGGS	PERV_Q4VFZ2_3mutA_WS
GSSGS	MLVMS_P03355_3mut
GSSGS	MLVAV_P03356_3mutA
GGSPAPGGG	MLVBM_Q7SVK7_3mutA_WS
GSSGGGGGS	MLVF5_P26810_3mut
PAPAPAPAP	MLVCB_P08361_3mut
PAPAP	PERV_Q4VFZ2_3mutA_WS
PAPGSSGS	KORV_Q9TTC1_3mut
PAPGSSGGG	PERV_Q4VFZ2_3mut
GGGEAAK	MLVMS_P03355_PLV919

Linker amino acid sequence	RT domain name
GGSGSGSGSGSGS	SFV3L_P27401-Pro_2mutA
GGSGGG	FLV_P10273_3mut
PAPEAAKGGG	MLVFF_P26809_3mut
PAP	PERV_Q4VFZ2_3mutA_WS
PAPGGSGSS	FFV_O93209_2mut
EAAAKEAAKEAAKEAAKEAAKEAAK	FFV_O93209-Pro_2mut
GSSGSSGSSGSS	FFV_O93209-Pro
GSSGSSGSSGSSGSS	FLV_P10273_3mutA
GGGEAAKPAP	PERV_Q4VFZ2
PAPGSSGGG	SFV3L_P27401_2mut
PAPGGSGSS	KORV_Q9TTC1-Pro_3mut
PAPAPAPAPAP	GALV_P21414_3mutA
GGSGGGEAAK	PERV_Q4VFZ2_3mut
GSSPAP	MLVCB_P08361_3mut
EAAKPAP	MLVF5_P26810_3mut
GGGGSGGGSGGGSGGGGS	MLVBM_Q7SVK7_3mut
GGSGGG	WMSV_P03359_3mut
GGSGSGGS	KORV_Q9TTC1_3mut
GGGGGGGG	MLVFF_P26809_3mut
GGGGSS	MLVAV_P03356_3mut
GSSGGGGGS	SFV3L_P27401_2mut
EAAAKEAAKEAAKEAAKEAAKEAAK	GALV_P21414_3mutA
GSSGSSGSS	PERV_Q4VFZ2_3mut
GSSPAPGGS	MLVFF_P26809_3mut
PAPAPAP	AVIRE_P03360_3mutA
EAAAKEAAKEAAKEAAK	WMSV_P03359_3mutA
PAPAPAPAP	SFV3L_P27401_2mutA
GGGGSS	MLVAV_P03356_3mutA
GSSGSSGSSGSSGSS	SFV3L_P27401_2mutA
PAPGGS	WMSV_P03359_3mutA
GSSEAAKGGG	PERV_Q4VFZ2
GSSGGSPAP	MLVMS_P03355_PLV919
GSSGSSGSSGSSGSSGSS	SFV3L_P27401_2mutA
GGSGSSGGG	MLVCB_P08361_3mut
GGGPAPGSS	SFV3L_P27401-Pro_2mutA
GSSEAAKGGG	WMSV_P03359_3mut
GSSEAAKGGG	MLVAV_P03356_3mut
GGSGGGPAP	FFV_O93209-Pro
GSSGSS	PERV_Q4VFZ2_3mut
PAPGGGGGS	GALV_P21414_3mutA

Linker amino acid sequence	RT domain name
EAAAKPAPGGS	MLVAV_P03356_3mut
GSSGSS	MLVMS_P03355_3mut
EAAAKPAPGGS	FFV_O93209-Pro
GGGPAPEAAK	MLVMS_P03355_3mutA_WS
GSSEAAKGGG	MLVBM_Q7SVK7_3mut
GGGEAAKGGG	BAEVM_P10272_3mut
GSSGSS	KORV_Q9TTC1-Pro_3mutA
EAAKEAAKEAAK	SFV1_P23074
PAPGSSGGS	KORV_Q9TTC1-Pro_3mut
PAPAPAPAPAP	MLVMS_P03355
GSSEAAK	SFV3L_P27401_2mut
PAP	PERV_Q4VFZ2_3mut
GSSEAAKGGG	MLVBM_Q7SVK7_3mut
GGSGGGPAP	MLVBM_Q7SVK7_3mutA_WS
GSSGSS	MLVMS_P03355_3mut
GSSEAAK	MLVMS_P03355
GSSEAAKGGG	MLVMS_P03355_PLV919
PAPGGGGGS	MLVFF_P26809_3mut
GSSGGG	PERV_Q4VFZ2_3mut
GSSGGS	PERV_Q4VFZ2_3mutA_WS
PAPGGG	BAEVM_P10272_3mut
PAPGSSGGG	MLVBM_Q7SVK7_3mut
GSSEAAK	SFV3L_P27401_2mut
GSSPAPEAAK	SFV3L_P27401-Pro_2mut
GSSGGSPAP	BAEVM_P10272_3mut
GGSPAPGSS	PERV_Q4VFZ2_3mutA_WS
GGSGSGGS	PERV_Q4VFZ2
GGSGGGPAP	FLV_P10273_3mut
GGGPAPEAAK	SFV3L_P27401_2mutA
GGGGS	FLV_P10273_3mutA
GSSGGSGGG	XMRV6_A1Z651_3mut
EAAKGGGGSS	PERV_Q4VFZ2
GGSGSSGGG	SFV3L_P27401-Pro_2mutA
GGSGSGGS	MLVFF_P26809_3mut
GGGPAPEAAK	FLV_P10273_3mut
GSSGGGEAAK	MLVMS_P03355_3mut
GGG	SFV3L_P27401_2mut
GSAGSAAGSGEF	WMSV_P03359_3mut
GSSGGGPAP	MLVMS_P03355_PLV919
GGGGSS	KORV_Q9TTC1-Pro_3mut

Linker amino acid sequence	RT domain name
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PAPGGSGGG	SFV3L_P27401_2mut
GSSGSSGSSGSSGSS	FFV_093209
GSSGGSPAP	MLVMS_P03355_3mut
GGSEAAK	KORV_Q9TTC1-Pro_3mutA
GGGGSGGGGS	BAEVM_P10272_3mut
GSSEAAKGGG	AVIRE_P03360_3mut
EAAKPAPGGG	FLV_P10273_3mut
EAAKGGSPAP	SFV3L_P27401-Pro_2mutA
GSSEAAKPAP	MLVBM_Q7SVK7_3mut
GGGPAPGGS	MLVCB_P08361_3mut
GGG	SFV3L_P27401_2mutA
EAAKGGGGSEAAK	SFV3L_P27401_2mutA
GGSGSSGGG	MLVBM_Q7SVK7_3mut
GSAGSAAGSGEF	BAEVM_P10272_3mut
GGGEAAK	FOAMV_P14350_2mutA
PAPEAAKGGG	WMSV_P03359_3mut
PAPAPAPAPAP	MLVF5_P26810_3mutA
GGSGGGGSS	FLV_P10273_3mutA
PAPGSSGGS	BAEVM_P10272_3mut
PAPEAAK	WMSV_P03359_3mutA
GSSGSSGSSGSSGSSGSS	FFV_093209-Pro_2mut
GGGGGSGSS	FFV_093209-Pro
GGGGGGGG	SFV3L_P27401-Pro_2mutA
GGGGGG	FLV_P10273_3mut
GSSGGSGGG	MLVAV_P03356_3mutA
GGGGSS	SFV3L_P27401-Pro_2mutA
GGSGGGPAP	FOAMV_P14350_2mut
GSSGSS	AVIRE_P03360_3mutA
EAAKEAAKEAAKEAAKEAAK	SFV3L_P27401-Pro_2mutA
EAAKEAAK	BAEVM_P10272_3mut
GSSPAPEAAK	GALV_P21414_3mutA
GGSEAAKPAP	SFV3L_P27401_2mutA
GGSGGGEAAK	SFV3L_P27401-Pro_2mutA
EAAKGGSPAP	FOAMV_P14350_2mut
GGSGSSEAAK	SFV3L_P27401_2mut
GGG	PERV_Q4VFZ2
GGGGGSGSS	FOAMV_P14350_2mut
GGSGGGEAAK	KORV_Q9TTC1-Pro_3mut
GSSGGSGGG	AVIRE_P03360_3mutA

Linker amino acid sequence	RT domain name
EAAAKPAPGGG	SFV3L_P27401_2mutA
PAPGGSGGG	KORV_Q9TTC1-Pro_3mut
PAPAPAP	WMSV_P03359_3mutA
GSSEAAKPAP	SFV1_P23074
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	SRV2_P51517
GSSGGSGGG	PERV_Q4VFZ2_3mutA_WS
GSSGSSGSSGSSGSSGSS	FFV_093209
GSSGGGPAP	WMSV_P03359_3mut
PAPAPAPAPAPAP	MLVBM_Q7SVK7_3mut
GGGGGSPAP	KORV_Q9TTC1-Pro_3mutA
PAPGSS	MLVBM_Q7SVK7_3mutA_WS
PAPEAAKGSS	SFV3L_P27401-Pro_2mut
GGGGSSPAP	MLVMS_P03355_3mut
GGSEAAK	FFV_093209-Pro
EAAAKPAPGSS	AVIRE_P03360_3mutA
PAPGSS	WMSV_P03359_3mut
PAPGGSGGG	SFV3L_P27401-Pro_2mutA
EAAAKEAAKEAAK	SFV3L_P27401_2mut
GGS	MLVRD_P11227_3mut
GGGS	KORV_Q9TTC1-Pro_3mut
GGSGGGGSS	KORV_Q9TTC1
GGSGGG	MLVMS_P03355_3mutA_WS
GGGEAAKPAP	BAEVM_P10272_3mut
EAAAKEAAKEAAKEAAKEAAK	FLV_P10273
PAPGGSGGG	KORV_Q9TTC1-Pro_3mutA
GSSGSSGSSGSSGSSGSS	HTL1L_P0C211
GGGEAAKPAP	WMSV_P03359
GSSGGSPAP	FFV_093209-Pro
PAPAPAPAPAP	SFV3L_P27401-Pro_2mutA
GSSGSEAAK	SFV3L_P27401_2mutA
GGSPAPGSS	SFV3L_P27401_2mut
GGSGSGGS	KORV_Q9TTC1-Pro_3mut
PAPEAAKGSS	KORV_Q9TTC1-Pro_3mut
EAAKGGS	KORV_Q9TTC1_3mutA
EAAKGGGGSEAAK	SFV3L_P27401-Pro_2mut
GGGGSSPAP	FFV_093209-Pro
EAAK	SFV3L_P27401_2mut
EAAKGGGGSS	BAEVM_P10272_3mut
GGGGSEAAK	MLVBM_Q7SVK7_3mut
GGGG	PERV_Q4VFZ2

Linker amino acid sequence	RT domain name
GGGGGSEAAK	FLV_P10273_3mut
EAAAKGGGPAP	KORV_Q9TTC1-Pro
GGGGSGGGSGGGSGGGGS	FFV_O93209_2mutA
GSSGGSGGG	PERV_Q4VFZ2_3mut
GGGGSGGGSGGGGS	GALV_P21414_3mutA
GGSGGGEAAK	AVIRE_P03360_3mutA
PAPEAAKGGG	SFV3L_P27401_2mut
GGGGSGGGGS	AVIRE_P03360
GSSGGGEAAK	SFV3L_P27401_2mutA
GGGGG	AVIRE_P03360_3mutA
GGSGSS	KORV_Q9TTC1_3mut
PAPAPAPAPAPAP	FOAMV_P14350_2mut
GGSEAAKPAP	KORV_Q9TTC1-Pro_3mut
GGGGGG	PERV_Q4VFZ2_3mut
GSSGGGEAAK	MLVBM_Q7SVK7
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVAV_P03356
GGSPAPGSS	BAEVM_P10272_3mut
GGGGSSPAP	BAEVM_P10272
GGGGSEAAKGGGGGS	SFV3L_P27401_2mut
GGGGGGGG	GALV_P21414_3mutA
PAPAP	MLVAV_P03356_3mut
GGGEAAK	PERV_Q4VFZ2_3mutA_WS
GSSPAPGGG	FFV_O93209_2mut
GGSGSGSGSGSGSGGS	BAEVM_P10272
GGGGGS	MLVF5_P26810_3mutA
PAPGGGGSS	FLV_P10273_3mutA
GGGEAAK	MLVBM_Q7SVK7_3mut
PAPEAAKGGG	WMSV_P03359_3mut
GSSEAAK	MLVBM_Q7SVK7_3mut
EAAAKEAAK	AVIRE_P03360
EAAAKGGGGGS	MLVBM_Q7SVK7_3mut
GGGEAAKGGG	SFV3L_P27401-Pro_2mutA
PAPAPAPAPAP	MLVF5_P26810_3mut
PAPGSSEAAK	SFV3L_P27401-Pro_2mutA
EAAAKEAAKEAAK	BAEVM_P10272_3mutA
GGSPAPGSS	MLVMS_P03355
PAPGSSGGG	FLV_P10273_3mutA
EAAAKEAAKEAAKEAAK	FOAMV_P14350-Pro_2mut
EAAAKGGG	KORV_Q9TTC1_3mutA
EAAAKGGSGGG	MLVBM_Q7SVK7_3mut

Linker amino acid sequence	RT domain name
GGGGGS	KORV_Q9TTC1-Pro_3mutA
PAPGGSGGG	WMSV_P03359_3mut
GGGPAPGGS	KORV_Q9TTC1_3mutA
GSS	FFV_093209
GGSGGSGGS	PERV_Q4VFZ2_3mut
GGGGS	GALV_P21414_3mutA
GGGG	MLVF5_P26810_3mut
GSSEAAKPAP	FFV_093209-Pro_2mut
PAPAPAPAP	FFV_093209-Pro
PAP	MLVF5_P26810_3mut
EAAAKEAAKEAAK	FFV_093209_2mut
EAAAKGSS	MLVCB_P08361_3mut
EAAAKGGG	MLVBM_Q7SVK7_3mut
PAPEAAKGGG	FFV_093209_2mut
GSSGGGEAAK	SFV1_P23074-Pro_2mut
PAPGGGEAAK	GALV_P21414_3mutA
GGGGSGGGSGGGSGGGGS	FOAMV_P14350-Pro_2mutA
GSSGGG	FOAMV_P14350_2mut
GGGGSGGGSGGGSGGGGS	SFV3L_P27401_2mutA
GSSGSS	AVIRE_P03360_3mut
GGSGSSEAAK	MMTVB_P03365_WS
PAPAPAP	MLVAV_P03356_3mutA
GSSGGSPAP	SFV3L_P27401-Pro_2mut
GGSPAP	AVIRE_P03360
GGSGGGPAP	FFV_093209
GSSEAAK	PERV_Q4VFZ2
GSSGGGPAP	PERV_Q4VFZ2_3mutA_WS
GGGGSSEAAK	KORV_Q9TTC1_3mutA
GSSEAAKPAP	SFVCP_Q87040
GGSGGGPAP	FOAMV_P14350_2mutA
GGGGSGGGSGGGSGGGGS	BLVJ_P03361_2mutB
GGGSSSPAP	SFV3L_P27401_2mutA
EAAAKGGS	MLVF5_P26810_3mut
GSSEAAKGSS	MLVCB_P08361_3mut
GGGGSSEAAK	SFV3L_P27401_2mut
EAAAKGGSGGG	FOAMV_P14350_2mut
GGSGGS	FLV_P10273_3mut
EAAAKGGG	FFV_093209-Pro
GSSGSSGSSGSSGSS	SFV3L_P27401
GSSGGGPAP	PERV_Q4VFZ2_3mutA_WS

Linker amino acid sequence	RT domain name
PAPGGSEAAK	SFV3L_P27401-Pro_2mutA
GGSPAP	KORV_Q9TTC1
EAAAKPAPGSS	KORV_Q9TTC1_3mutA
SGSETPGTSESATPES	SFV1_P23074
GSSPAP	SFV3L_P27401-Pro_2mutA
GSSPAPGGG	SFV3L_P27401_2mut
GGGEAAKGSS	SFV1_P23074_2mut
GGGPAPGGS	BAEVM_P10272_3mut
EAAAKGGG	KORV_Q9TTC1-Pro_3mutA
GSSGGG	SFV3L_P27401-Pro_2mut
GGSPAPEAAK	BAEVM_P10272_3mut
EAAAKGSSPAP	FFV_093209
EAAAKGGGSEAAK	SFV3L_P27401-Pro_2mutA
GSSGSSGSSGSSGSS	SFV1_P23074_2mut
EAAAKGGSPAP	FOAMV_P14350_2mut
GGSGGS	KORV_Q9TTC1-Pro_3mutA
EAAAKGSSGGS	GALV_P21414
GSSGGGPAP	MLVAV_P03356
PAPEAAKGGS	FOAMV_P14350_2mut
EAAAKPAPGGG	AVIRE_P03360_3mut
GGSPAP	SFV3L_P27401_2mutA
GGGGSGGGGS	SFV3L_P27401_2mutA
GGGGSS	AVIRE_P03360_3mutA
GGSPAPGGG	SFV3L_P27401-Pro_2mutA
EAAAKPAPGSS	SFV3L_P27401
EAAAKPAP	FOAMV_P14350-Pro_2mut
PAPEAAKGGS	PERV_Q4VFZ2_3mutA_WS
EAAAKGGSGSS	SFV3L_P27401_2mutA
GGGEAAKGSS	GALV_P21414_3mutA
GGGGSEAAKGGGGS	PERV_Q4VFZ2_3mut
PAPGGSGSS	FFV_093209-Pro_2mutA
GGSEAAKPAP	GALV_P21414_3mutA
GGSGSGSGSGSGSGS	FFV_093209-Pro
GSSGGSEAAK	SFV3L_P27401-Pro_2mut
GGS	GALV_P21414_3mutA
PAPGGSEAAK	MLVMS_P03355
PAPEAAKGGS	BAEVM_P10272_3mutA
GGSGSSPAP	SFV3L_P27401-Pro_2mutA
GSSPAP	WMSV_P03359_3mut
GGGEAAK	MMTVB_P03365

Linker amino acid sequence	RT domain name
GGGGSS	PERV_Q4VFZ2_3mut
GGSPAPGSS	SFV3L_P27401-Pro_2mut
PAPGGS	MLVBM_Q7SVK7_3mut
EAAAKGSSPAP	MLVBM_Q7SVK7_3mut
GGGGSSGGSS	PERV_Q4VFZ2_3mut
PAPAPAPAPAPAP	SFV1_P23074
GGSEAAAKGGG	SFV3L_P27401-Pro_2mut
GGSSGGSS	SFV1_P23074_2mut
GSSGGGGSS	MLVF5_P26810_3mutA
EAAAKGGGPAP	SFV3L_P27401
EAAAKEAAKEAAKEAAK	FOAMV_P14350-Pro_2mutA
GGGPAPGSS	SFV3L_P27401_2mutA
GGGSGGGSGGGSGGGSS	SFV3L_P27401_2mut
EAAKEAAKEAAKEAAK	MMTVB_P03365_WS
PAPGSSGGSS	KORV_Q9TTC1-Pro_3mutA
PAPGSSEAAK	FOAMV_P14350-Pro_2mut
GSSPAPEAAK	BAEVM_P10272_3mut
EAAKGGGSEAAK	FFV_O93209-Pro
GGSPAP	PERV_Q4VFZ2
GGSGSSEAAK	XMRV6_A1Z651_3mut
GGSEAAAKGGG	GALV_P21414_3mutA
PAPGGGGSS	AVIRE_P03360_3mutA
GGSGSGSGSGSS	PERV_Q4VFZ2
GGGGSSGGSS	PERV_Q4VFZ2_3mutA_WS
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	BAEVM_P10272_3mutA
GGGPAP	MLVAV_P03356_3mut
GGGSGGGSGGGSGGGSS	FFV_O93209_2mut
GSSEAAK	FFV_O93209
GGSPAPEAAK	FOAMV_P14350_2mut
GGGGGSEAAK	FOAMV_P14350_2mut
GSSPAPGGS	MLVBM_Q7SVK7_3mut
GSS	SFVCP_Q87040_2mut
EAAAKPAP	FOAMV_P14350-Pro
EAAAKGGG	SFV3L_P27401_2mut
GGGEAAK	AVIRE_P03360_3mutA
PAPGSSGGG	WMSV_P03359_3mut
EAAAKGGSPAP	SFV3L_P27401
GSSGGSGGG	SFV3L_P27401-Pro_2mutA
GSSGGGEAAK	GALV_P21414_3mutA
GGGPAPGSS	MLVBM_Q7SVK7_3mutA_WS

Linker amino acid sequence	RT domain name
PAPGGGEAAK	FFV_093209-Pro_2mut
GSSGSSGSSGSS	SFV1_P23074_2mut
GGSEAAK	PERV_Q4VFZ2_3mutA_WS
GGGEAAKPAP	SFV3L_P27401_2mut
EAAKGGGPAP	SFV3L_P27401_2mut
GGGGSSPAP	FLV_P10273_3mut
EAAKPAPGSS	FFV_093209_2mut
GGGGSSPAP	SFV3L_P27401_2mut
GSSGSS	KORV_Q9TTC1_3mutA
GGGGSGGGSGGGSGGGSGGGGS	BLVJ_P03361_2mut
GGGGSSGGS	GALV_P21414_3mutA
EAAKGGSGSS	FFV_093209-Pro
EAAKPAP	PERV_Q4VFZ2
GSSGGGEAAK	MLVBM_Q7SVK7_3mut
PAPGGSGGG	BAEVM_P10272
EAAKGGGPAP	MLVF5_P26810
GSSGSSGSS	MLVBM_Q7SVK7_3mut
GSSGGS	AVIRE_P03360_3mutA
GGSEAAKGGG	FOAMV_P14350_2mut
EAAKGGG	MLVF5_P26810_3mutA
GGSGSSGGG	WMSV_P03359_3mut
EAAK	SFV1_P23074_2mut
GSSGGSPAP	SFV3L_P27401-Pro_2mutA
GGGGSSGGS	KORV_Q9TTC1_3mut
PAPGGSGGG	FFV_093209-Pro_2mut
GGGPAPGGS	SFV3L_P27401_2mutA
GSSPAPEAAK	FLV_P10273_3mut
GGSGSSPAP	SFV3L_P27401_2mut
GSSEAAKGGG	SFV3L_P27401_2mut
PAPGGG	SFV3L_P27401_2mutA
SGSETPGTSESATPES	KORV_Q9TTC1-Pro_3mut
GGGGS	SFV1_P23074-Pro_2mutA
GSSGGGEAAK	WMSV_P03359
EAAKGGGGSEAAK	MLVF5_P26810_3mutA
GSSEAAKPAP	FFV_093209
GGGGGG	SFV1_P23074_2mutA
EAAKEAAKEAAK	MMTVB_P03365-Pro
EAAKPAPGSS	MLVBM_Q7SVK7_3mut
GGSGSSEAAK	SFV3L_P27401_2mutA
GGSEAAK	MLVMS_P03355_3mut

Linker amino acid sequence	RT domain name
GGSPAPEAAAK	SFV3L_P27401_2mut
GGGPAPGSS	SFV1_P23074
GGGGGSEAAAK	MLVBM_Q7SVK7_3mutA_WS
EAAAKPAPGSS	KORV_Q9TTC1-Pro
GSSGSSGSSGSS	SFV3L_P27401_2mut
EAAAKPAP	SFV3L_P27401_2mut
GGGEAAAK	PERV_Q4VFZ2_3mut
GSSGSS	SFV3L_P27401_2mutA
EAAAKGSSGSS	MMTVB_P03365
SGSETPGTSESATPES	SFV3L_P27401
EAAAKGSSGGG	PERV_Q4VFZ2
EAAAKEAAKEAAKEAAKEAAKEAAK	MMTVB_P03365
GSSGGGPAP	KORV_Q9TTC1_3mutA
PAPAPAPAP	SFV3L_P27401
GGGEAAAKGSS	SFV1_P23074_2mut
GSSGGSGGG	PERV_Q4VFZ2_3mut
PAPEAAAKGSS	FOAMV_P14350_2mutA
GGGEAAAKGSS	SFV3L_P27401_2mut
GGGGSGGGSGGGSGGGSS	MLVBM_Q7SVK7
PAPGSSGGG	FLV_P10273
GSSGSSGGG	FFV_093209
EAAAKPAPGSS	MLVBM_Q7SVK7
GSSEAAAKGGG	SFV3L_P27401_2mutA
GSSGGSGSGSGSGSS	MLVF5_P26810
GSSEAAKPAP	SFV3L_P27401-Pro_2mutA
EAAAKGGSPAP	SFV3L_P27401_2mutA
EAAAKGGGGSS	SFV3L_P27401_2mut
GSSPAPEAAAK	SFV3L_P27401_2mutA
PAPAP	MLVBM_Q7SVK7_3mut
PAPGGSEAAAK	KORV_Q9TTC1-Pro
GSSGSS	MLVF5_P26810_3mutA
GSSEAAKPAP	FFV_093209_2mut
GSS	MLVMS_P03355
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	SFV3L_P27401-Pro
PAPGGGEAAAK	SFV3L_P27401_2mut
PAPGGGGSS	SFV3L_P27401-Pro_2mut
PAPGGSGSS	BAEVM_P10272_3mut
GSSGGGEAAAK	FFV_093209
GSSEAAKPAP	SFV1_P23074_2mut
GGGGG	FLV_P10273_3mut

Linker amino acid sequence	RT domain name
GGGEAAKGSS	SFV3L_P27401
GSSGSSGSSGSSGSS	SFV1_P23074-Pro
SGSETPGTSESATPES	AVIRE_P03360
PAPGSSGGG	MLVBM_Q7SVK7_3mut
GGGGSSPAP	HTL3P_Q4U0X6_2mut
GGGEAAK	SFV1_P23074
GGSGGG	AVIRE_P03360
EAAAKGSSGGG	SFV3L_P27401_2mutA
GSSPAPEAAK	FOAMV_P14350-Pro_2mutA
GGGPAPGSS	WMSV_P03359
EAAAKGSSGGG	MLVMS_P03355
GGGGSEAAK	MLVMS_P03355
EAAKPAPGSS	SFV3L_P27401
EAAKGSSPAP	SFV3L_P27401
GGGGGGG	FOAMV_P14350_2mutA
EAAKEAAKEAAK	SFV3L_P27401
GSSPAPGSS	FFV_O93209_2mutA
GGGSSEAAK	SFV3L_P27401-Pro_2mutA
GGSEAAKGSS	GALV_P21414_3mutA
GGSEAAKGSS	BAEVM_P10272_3mutA
EAAKPAPGGG	MLVCB_P08361
GSSGSSGSSGSSGSSGSS	SFV1_P23074-Pro
GGGGSEAAKGGGGS	FOAMV_P14350_2mut
GSSPAPGSS	MLVMS_P03355_PLV919
GGGGSGGGGS	FFV_O93209-Pro
GSSGGSPAP	KORV_Q9TTC1_3mutA
GGSGGS	GALV_P21414_3mutA
PAPGSSEAAK	WMSV_P03359
PAPGGGGSS	MMTVB_P03365-Pro
GGGGSSGGS	PERV_Q4VFZ2_3mutA_WS
GGGGSGGGGS	FFV_O93209_2mut
GGGGSGGGSGGGSGGGGS	XMRV6_A1Z651
GGGSSEAAK	SFV1_P23074_2mut
GGSGGGGSS	GALV_P21414_3mutA
GGSEAAKPAP	MLVBM_Q7SVK7
EAAKGSPAP	SFV1_P23074_2mutA
PAPAPAPAP	FFV_O93209
GSSGGSPAP	MMTVB_P03365-Pro
GGGGGSPAP	KORV_Q9TTC1_3mutA
EAAKGGPAP	PERV_Q4VFZ2

Linker amino acid sequence	RT domain name
GSSGGSPAP	BAEVM_P10272
GGGGG	FFV_093209
GGGGGS	FLV_P10273_3mutA
EAAAKEAAKEAAK	FOAMV_P14350
PAPGGG	MLVCB_P08361_3mut
GSSGGSEAAK	FOAMV_P14350_2mutA
GGSPAPGGG	FLV_P10273_3mut
GSSGGSSGSSGSSGSS	SFV1_P23074-Pro_2mutA
GGSPAPEAAK	SFV3L_P27401
PAPGGGGSS	HTL3P_Q4U0X6_2mutB
GGGGSEAAK	MMTVB_P03365_2mut_WS
PAPGGS	MLVRD_P11227_3mut
GSSGGSGGSGGSGGS	MMTVB_P03365
GSAGSAAGSGEF	AVIRE_P03360
GSSGGS	BAEVM_P10272_3mutA
GGSGGGGSS	MMTVB_P03365
GGSGGGGSS	WMSV_P03359
PAPEAAKGSS	SFV1_P23074
GSSGGSSGSSGSS	SFV1_P23074-Pro_2mutA
PAPAPAPAPAPAP	SFV3L_P27401
PAPGSSGGG	FLV_P10273_3mut
GGSGSSPAP	MLVMS_P03355
GGSGGGPAP	FOAMV_P14350
PAPGGGGGS	KORV_Q9TTC1_3mutA
EAAAKGSSPAP	GALV_P21414_3mutA
GGSGSSPAP	MLVBM_Q7SVK7_3mut
EAAAKGSS	SFV3L_P27401_2mut
GGGGSEAAK	WMSV_P03359
GGGGGGGG	SFV1_P23074-Pro
EAAAKEAAK	MLVBM_Q7SVK7
GGGEAAKGGS	MLVBM_Q7SVK7
EAAAKGSSPAP	SFV3L_P27401_2mut
GSSEAAK	XMRV6_A1Z651
PAPGGGEAAK	MMTVB_P03365_WS
GGSPAP	GALV_P21414_3mutA
GSSPAPGGG	MLVBM_Q7SVK7_3mutA_WS
GGSGSSPAP	SFV1_P23074_2mutA
GGS	HTL32_Q0R5R2_2mut
GGSGGGGSS	MMTVB_P03365-Pro
GGGGSGGGSGGGSGGGGS	SFVCP_Q87040_2mutA

Linker amino acid sequence	RT domain name
EAAAKGGGPAP	FOAMV_P14350_2mut
GSSGGGEAAAK	MMTVB_P03365
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVBM_Q7SVK7_3mutA_WS
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	MMTVB_P03365_WS
EAAAKEAAK	FOAMV_P14350-Pro_2mut
GSSPAPEAAK	FOAMV_P14350_2mutA
EAAAKPAPGSS	GALV_P21414_3mutA
GSSGGSPAP	KORV_Q9TTC1-Pro_3mut
GGGPAPEAAK	MLVAV_P03356
GGGEAAKPAP	SFV1_P23074-Pro_2mut
GGGGGSEAAK	SFV3L_P27401_2mut
GGGPAPGSS	SFV3L_P27401_2mut
GGSEAAKPAP	AVIRE_P03360
GSSGSSGSSGSSGSSGSS	SFV1_P23074-Pro_2mut
EAAAKGSSGSS	FOAMV_P14350_2mutA
GGGGGG	MLVBM_Q7SVK7_3mut
GSSPAPGSS	PERV_Q4VFZ2
GGSGSSPAP	GALV_P21414_3mutA
GGGPAPEAAK	SFV3L_P27401
GGSGGGEAAK	WMSV_P03359
GSAGSAAGSGEF	SFV1_P23074_2mut
GSSGGGEAAK	MLVMS_P03355
GGG	MMTVB_P03365-Pro
PAPGSSGSS	FOAMV_P14350_2mut
GGGGSSPAP	FFV_O93209_2mut
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MMTVB_P03365_WS
GGGGGGG	XMRV6_A1Z651
PAPAPAPAPAP	FOAMV_P14350
GGGGSGGGSGGGSGGGSS	MMTVB_P03365_2mut_WS
GGSGGPAP	SFV3L_P27401_2mut
GGGGGG	SFV1_P23074-Pro
EAAAKPAPGSS	SFV3L_P27401_2mut
GGGGSSGSS	HTL3P_Q4U0X6_2mut
PAPGSSEAAK	MMTVB_P03365-Pro
GGGGSSPAP	FOAMV_P14350-Pro_2mut
PAPGSSGSS	MMTVB_P03365
AEEAAKEAAKEAAKEAAKALEAEEAAKEAAKEAAKEAAKA	SRV2_P51517
PAPAPAP	MMTVB_P03365_2mut_WS
PAPGGGGSS	MMTVB_P03365_2mutB
GGGGSS	SFV1_P23074-Pro_2mutA

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Linker amino acid sequence	RT domain name
EAAAKEAAKEAAKEAAKEAAKEAAK	SRV2_P51517
GGGGSGGGSGGGSGGGSGGGSGGGGS	MMTVB_P03365
GGGEAAKGGG	MMTVB_P03365_WS
GSSGSS	SFV1_P23074
GSSGGGGG	SFV3L_P27401
GGGGSSEAAK	SFV1_P23074
EAAKGGSSGG	HTL1A_P03362_2mutB
GSSEAAKGGG	GALV_P21414_3mutA
EAAKGGSPAP	SFV1_P23074
EAAKPAPGSS	SFV3L_P27401_2mutA
PAPGSSGGG	SFV3L_P27401-Pro_2mut
GGGGSGGGSGGGSGGGSGGGSGGGGS	SFV3L_P27401-Pro
EAAKEAAKEAAKEAAKEAAK	MMTVB_P03365_WS
GGGGSSEAAK	MLVF5_P26810_3mutA
EAAKGGSPAP	GALV_P21414
PAPEAAKGGG	MMTVB_P03365_WS
GSSGGGGG	SFVCP_Q87040_2mut
GGGGSSPAP	SFV1_P23074
EAAKGGGGSS	XMRV6_A12651
PAPAPAPAP	MMTVB_P03365
GGSEAAKGGG	SFV3L_P27401_2mutA
GSSPAPGGG	MMTVB_P03365_WS
GGGGGG	SFV3L_P27401-Pro
GGSGGGGG	FOAMV_P14350-Pro_2mut
PAPAPAPAPAPAP	WMSV_P03359
GSSPAP	MLVBM_Q7SVK7
GGGGSGSS	MMTVB_P03365_2mut_WS
EAAKGGSSGG	MMTVB_P03365_2mutB_WS
EAAK	FFV_O93209_2mutA
PAPEAAK	SFV1_P23074-Pro
EAAKGGSGSS	SFV3L_P27401
GGSGGGGG	FFV_O93209-Pro
GSSGGGEAAK	MMTVB_P03365
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MLVFF_P26809_3mutA
GGSGGGGGSGGGSGGGG	HTL1L_P0C211_2mutB
GGGEAAK	SFV3L_P27401-Pro_2mutA
GGGGSGSS	MMTVB_P03365
GSSPAPGGG	FOAMV_P14350_2mutA
EAAKGGSS	MLVMS_P03355
GSSGGSGGG	FFV_O93209-Pro

Linker amino acid sequence	RT domain name
GGSGGGSS	MMTVB_P03365-Pro_2mut
GGSPAPGSS	FOAMV_P14350_2mut
GGSGSGSGSGSGSGSS	SFVCP_Q87040-Pro_2mut
GSSEAAKGGG	FOAMV_P14350_2mutA
GGSGSGSGS	MMTVB_P03365-Pro
GSSGSSGSSGSSGSSGSS	MMTVB_P03365_2mut_WS
GSSGSSGSSGSSGSS	MMTVB_P03365-Pro
PAPEAAK	WDSV_Q92815
GSSGSSGSSGSSGSS	FFV_Q93209-Pro_2mut
EAAKGGGSEAAK	MMTVB_P03365-Pro
GGSPAPEAAK	FOAMV_P14350
GSSGSS	PERV_Q4VFZ2
GGG	MMTVB_P03365-Pro
GGGGSGGGSGGGSS	FFV_Q93209_2mut
EAAKEAAKEAAKEAAKEAAKEAAK	MMTVB_P03365-Pro
GGSGSSPAP	WMSV_P03359
GGGGGGGG	SFV3L_P27401_2mut
PAPGSSEAAK	FOAMV_P14350-Pro_2mutA
GGGGSSPAP	FOAMV_P14350_2mut
GSSGGSPAP	MLVBM_Q7SVK7_3mut
GSSGGGGSS	GALV_P21414_3mutA
EAAKEAAKEAAKEAAKEAAKEAAK	MMTVB_P03365
GSSGGGGSS	SFV1_P23074_2mut
GGGGSEAAKGGGGSS	SFV1_P23074
GGGEAAKPAP	FFV_Q93209
PAPGGGEAAK	SFV1_P23074
GGSGGGEAAK	PERV_Q4VFZ2_3mutA_WS
GSSGGG	MMTVB_P03365-Pro
EAAKGGSSGSS	FFV_Q93209_2mut
GGGGG	SFV1_P23074_2mut
GGGPAP	SFV3L_P27401
GSSGGSEAAK	FFV_Q93209
SGGSSGGSSGSETPGTSESATPESSGGSSGSS	MMTVB_P03365-Pro
GSSGGGEAAK	SFV1_P23074_2mutA
GSSGSSGSSGSSGSS	SFV3L_P27401_2mut
GGSEAAKPAP	FLV_P10273
GGGGSGGGSS	FOAMV_P14350-Pro_2mutA
GSSEAAKPAP	SFV3L_P27401
GGGGSEAAKGGGGSS	MMTVB_P03365-Pro
PAPGSSEAAK	MLVF5_P26810_3mut

Linker amino acid sequence	RT domain name
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GGGPAPGGS	SFV3L_P27401
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GSSGGSPAP	SFV3L_P27401_2mutA
PAPAP	FFV_093209
PAPGGSGGS	MMTVB_P03365_WS
EAAAKGGGGGS	FOAMV_P14350_2mut
PAPEAAKGGS	SFV3L_P27401_2mut
GSSEAAKPAP	MMTVB_P03365-Pro
GSSGGS	PERV_Q4VFZ2_3mut
GSSEAAKGGG	FFV_093209-Pro_2mutA
EAAAK	HTL1L_P0C211
GSSPAP	MLVMS_P03355
EAAKPAPGGG	FFV_093209-Pro_2mut
GGGGSEAAKGGGGS	SFV1_P23074-Pro_2mut
EAAAKGSSGGS	SFV3L_P27401
GSAGSAAGSGEF	FFV_093209_2mutA
PAPEAAKGGS	MMTVB_P03365_2mutB_WS
EAAKEAAKEAAKEAAKEAAKEAAK	MMTVB_P03365
GGS	MMTVB_P03365
GGSEAAKPAP	SFV1_P23074
EAAKGSSGGG	HTLV2_P03363_2mut
GGSEAAKGGG	MMTVB_P03365_WS
GSSGGS	FFV_093209-Pro
GSSEAAKGGS	MMTVB_P03365-Pro
PAPAPAPAPAP	SFV1_P23074_2mutA
GGSEAAKGGG	MMTVB_P03365_2mutB_WS
PAPAPAPAP	MMTVB_P03365_WS
GGGGSGGGSGGGSGGGSGGGGS	HTL3P_Q4U0X6_2mut
PAPGGSEAAK	SFV1_P23074-Pro_2mut
GSSGGGPAP	MMTVB_P03365
GSSGSSGSSGSSGSSGSS	MMTVB_P03365-Pro
GGSEAAKPAP	SFV1_P23074-Pro
GGGEAAKGSS	SFV3L_P27401_2mutA
GGGPAPGGS	AVIRE_P03360
PAPGGG	MLVRD_P11227
GGSEAAKGSS	SFV3L_P27401_2mut
GGGEAAKGSS	FOAMV_P14350_2mut
GGGEAAKGSS	SFV1_P23074-Pro

Linker amino acid sequence	RT domain name
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EAAAKGGGPAP	JSRV_P31623_2mutB
EAAAKGGGGSS	FOAMV_P14350_2mut
EAAKEAAKEAAKEAAKEAAK	SRV2_P51517
GSSGGGGSS	FFV_093209
PAPAPAP	FOAMV_P14350_2mutA
GGSGSGSGSGSS	FOAMV_P14350
GGGEAAK	MMTVB_P03365_WS
GGGGSS	SFV1_P23074_2mutA
GGSGGS	WMSV_P03359_3mut
EAAAKGGS	MMTVB_P03365-Pro
GGGGSS	BLVJ_P03361_2mut
PAPAP	MMTVB_P03365-Pro_2mut
PAPGGG	SMRVH_P03364
EAAAKGGGGSS	SFV3L_P27401
PAPAPAPAPAP	MMTVB_P03365
GGGPAP	MMTVB_P03365-Pro
GSSGGSGGG	MMTVB_P03365
EAAAKGGGPAP	FOAMV_P14350_2mutA
GSSGSSGSSGSS	SFV1_P23074
GGGGSGGGSS	SFV3L_P27401
GSSGGSGGG	MLVF5_P26810
GGGEAAKPAP	MMTVB_P03365-Pro
PAPEAAK	HTLV2_P03363_2mut
GSSGSSGSSGSS	FOAMV_P14350_2mut
GSSEAAKPAP	MMTVB_P03365-Pro
PAPEAAKGGG	HTLV3P_Q4U0X6_2mut
GSSEAAKGSS	MMTVB_P03365-Pro
EAAAKPAPGGS	MMTVB_P03365_2mut_WS
GSSGSEAAK	MLVF5_P26810_3mutA
GGGGSGGGSGGGSGGGSGGGSGGGSS	MLVF5_P26810_3mut
EAAAKGGGGSS	MMTVB_P03365-Pro
GGGGSGSS	HTLV1A_P03362_2mutB
PAPAP	FFV_093209-Pro_2mut
GGGGSPAP	HTLV1C_P14078_2mut
GGGPAP	HTLV2_P03363_2mut
EAAAKGGGSEAAK	SFVCP_Q87040
GSSEAAKGGG	FFV_093209-Pro_2mutA
GSSPAPGGS	FOAMV_P14350-Pro_2mut
GGGGGGG	MMTVB_P03365-Pro

Linker amino acid sequence	RT domain name
EAAAKGSS	SFV3L_P27401_2mutA
EAAAKGGGSEAAK	MMTVB_P03365-Pro
GGGGSEAAKGGGS	SFV1_P23074-Pro_2mutA
EAAAKGGGSS	MMTVB_P03365
GGGEAAKGGG	SFV1_P23074
PAPEAAKGGG	MLVF5_P26810
GGGGSSGGG	MMTVB_P03365
GGSGSS	MMTVB_P03365
PAPAPAPAPAP	KORV_Q9TTC1
EAAAKGGG	SFV1_P23074-Pro_2mut
PAPAPAPAPAP	SRV2_P51517
GSSGSSGSSGSSGSS	FFV_O93209-Pro_2mutA
GGGGSS	FOAMV_P14350_2mut
PAPGGGEAAK	MMTVB_P03365_WS
GGSGGGEAAK	FFV_O93209-Pro_2mut
PAPAPAPAPAP	MMTVB_P03365_WS
GGGEAAKGGG	MMTVB_P03365-Pro
GGGEAAKGGSS	MMTVB_P03365_2mutB
GSSPAPEAAK	MMTVB_P03365_WS
EAAKEAAKEAAKEAAKEAAK	SFV1_P23074-Pro_2mutA
PAPGGG	SFV3L_P27401
GSSEAAKGGG	MMTVB_P03365_WS
GGGGSSEAAK	FOAMV_P14350_2mut
PAPGSSGGG	SFV1_P23074-Pro_2mut
GSSGSSGSSGSSGSSGSS	SFV3L_P27401
EAAAKGSSGGG	MMTVB_P03365
PAPGGGGSS	WDSV_O92815_2mutA
GGSPAP	MMTVB_P03365-Pro
GGSGGSGGSGGSGGSS	SFVCP_Q87040-Pro_2mut
PAPAPAPAP	MMTVB_P03365-Pro
GGGGG	HTL1A_P03362
GGSGGSGGSGGSS	SFV1_P23074_2mutA
GSSGSSGSSGSSGSSGSS	FOAMV_P14350-Pro_2mut
PAPGGSEAAK	MMTVB_P03365_2mutB_WS
PAPAPAPAP	SFV1_P23074_2mut
PAPGGGGSS	MMTVB_P03365
GGSGSS	SFV3L_P27401_2mut
EAAKEAAKEAAKEAAKEAAK	MMTVB_P03365_2mut
EAAAKGGSGGG	HTL3P_Q4U0X6_2mut
PAPGGGGSS	SFVCP_Q87040-Pro_2mutA

Linker amino acid sequence	RT domain name
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GGGGGS	FOAMV_P14350_2mut
GGGEAAAKGGS	FFV_O93209-Pro_2mutA
EAAAKPAPGGG	MMTVB_P03365_2mutB
GGSGGGPAP	FFV_O93209_2mut
GSSEAAKPAP	MMTVB_P03365
PAPAPAPAPAPAP	SFV1_P23074_2mut
GGSPAPGGG	MMTVB_P03365-Pro
GGSGGGEAAAK	MMTVB_P03365
PAPAP	SFVCP_Q87040
GSSEAAAK	SFVCP_Q87040
GGGGSGGGSGGGGS	MMTVB_P03365-Pro
GSSGSSGSS	SFV3L_P27401
EAAAKGGSGGG	MMTVB_P03365-Pro
GSSPAP	SFV1_P23074_2mut
GGGEAAAK	SFV1_P23074-Pro
AEEAAKEAAAKEAAAKEAAAKALEAAAKEAAAKEAAAKEAAAKA	MMTVB_P03365-Pro
PAPGGS	HTL1C_P14078_2mut
PAPGSSGGS	SFV1_P23074_2mut
PAPEAAAK	MMTVB_P03365_WS
PAPAP	MMTVB_P03365-Pro
EAAAKGGS	HTL1A_P03362_2mut
GGGGEAAAKGGGGS	HTL1C_P14078
EAAAKGSSGGS	FOAMV_P14350-Pro
PAPGGSGSS	MMTVB_P03365-Pro
PAPGGSEAAAK	SFV1_P23074_2mut
PAPGSSEAAAK	FFV_O93209-Pro_2mut
PAPGSSGGG	FOAMV_P14350-Pro_2mutA
GSSGGGEAAAK	AVIRE_P03360
GGGGGG	SMRVH_P03364_2mut
PAPEAAAKGGG	MMTVB_P03365-Pro
GGGEAAAKGGS	SFVCP_Q87040_2mutA
PAPAPAPAPAP	SRV2_P51517
GSSGSSGSSGSSGSSGSS	MMTVB_P03365
EAAAKGGGPAP	MLVAV_P03356
PAPAPAPAPAP	FOAMV_P14350-Pro_2mutA
PAPGGSEAAAK	FOAMV_P14350
GSSGGGPAP	HTL32_Q0R5R2_2mutB
GGGGGSPAP	HTL3P_Q4U0X6_2mutB
GSSGGSGGG	MMTVB_P03365-Pro

Linker amino acid sequence	RT domain name
PAPAP	SFVCP_Q87040-Pro
GSSGGGPAP	MMTVB_P03365-Pro
GGSGSS	MMTVB_P03365-Pro_2mut
GGSPAPEAAK	SFV1_P23074-Pro_2mut
EAAKGGSGGG	SFV3L_P27401_2mut
GGGGSSEAAK	MMTVB_P03365_WS
GGGGSGSGS	MMTVB_P03365_2mut
GGGGSSGGS	SFV1_P23074-Pro_2mutA
EAAKGGGGSEAAK	MMTVB_P03365_WS
PAPGGGEAAK	SFV1_P23074-Pro
PAPEAAKGGG	MMTVB_P03365
AEEAAKEAAKEAAKEAAKALEAAKEAAKEAAKEAAKEAAKA	MMTVB_P03365
GSSGGSEAAK	FOAMV_P14350-Pro_2mut
GGSPAP	MLVBM_Q7SVK7_3mut
GSSEAAK	FOAMV_P14350
GSSEAAK	MMTVB_P03365-Pro
EAAKGGSGGS	HTL1A_P03362_2mut
GGGEAAKPAP	FOAMV_P14350-Pro_2mut
EAAKGGSPAP	FOAMV_P14350
GSSEAAKPAP	MMTVB_P03365_WS
GSSGSSGSS	FOAMV_P14350_2mut
EAAKEAAKEAAKEAAK	MMTVB_P03365_WS
EAAK	MMTVB_P03365
PAPGSS	BAEVM_P10272
PAPGGS	FFV_O93209-Pro_2mut
GGSGGS	SFV1_P23074-Pro_2mutA
SGGSSGSSGSETPGTSESATPESSGGSSGGSS	HTLV2_P03363_2mut
GGSGGGEAAK	MMTVB_P03365_WS
PAPGSSGGG	HTL1A_P03362
GGSGGS	SFV3L_P27401-Pro
GSSGSS	SFV1_P23074-Pro
PAPGGSEAAK	MMTVB_P03365
GSAGSAAGSGEF	MMTVB_P03365-Pro
PAPGGG	FOAMV_P14350_2mut
EAAKGGSGSS	MMTVB_P03365_WS
GSSGGGEAAK	SFV3L_P27401-Pro
GGSGGPAP	FOAMV_P14350-Pro_2mut
PAPAPAPAPAPAP	WDSV_O92815
SGSETPGTSESATPES	SFVCP_Q87040-Pro_2mutA
GGSGSGGS	SFV1_P23074

Linker amino acid sequence	RT domain name
GGGGSS	SFVCP_Q87040_2mut
GGGGSEAAK	MMTVB_P03365
SGSETPGTSESATPES	MMTVB_P03365_WS
PAPAPAP	SFV3L_P27401
PAPEAAKGSS	MMTVB_P03365_2mutB_WS
GSSGSSGSSGSSGSS	SRV2_P51517
GGGPAPGSS	HTL32_Q0R5R2_2mutB
GSSGGGGSS	MMTVB_P03365-Pro
SGSETPGTSESATPES	SRV2_P51517
EAAKGSSGSS	MMTVB_P03365-Pro
GSSPAPEAAK	MMTVB_P03365-Pro
GSSPAPEAAK	SRV2_P51517
GGGSSSPAP	MMTVB_P03365-Pro
PAPGGGEAAK	SFV1_P23074-Pro_2mutA
PAPEAAKGSS	MMTVB_P03365
GSSGSSGSSGSSGSSGSS	FOAMV_P14350-Pro
GGSPAPGSS	SFV3L_P27401
GGGPAPGSS	SFV1_P23074-Pro_2mutA
GGGPAPGSS	MMTVB_P03365-Pro
EAAKPAP	MLVBM_Q7SVK7
EAAKEAAKEAAK	HTL1C_P14078
GSSGGSEAAK	SRV2_P51517
PAPGGGGSS	SRV2_P51517
GGGEAAK	FFV_O93209-Pro_2mut
EAAKGGGPAP	HTL32_Q0R5R2
GSSGSSGGG	MMTVB_P03365
PAPEAAKGSS	MMTVB_P03365-Pro
PAPGGGGSS	MMTVB_P03365-Pro
EAAKGGGGSS	MMTVB_P03365_WS
GGGGGS	MMTVB_P03365-Pro
GGGGSGGGSGGGSGGGSGGGGS	HTL1C_P14078
EAAKGGSPAP	MMTVB_P03365
GGGSSSPAP	FFV_O93209-Pro_2mut
GGGSSSGGS	MMTVB_P03365-Pro
PAPGSSGGS	MMTVB_P03365-Pro
GGGGGS	SRV2_P51517
GSSGSSGGG	MMTVB_P03365
GSSGGSEAAK	MMTVB_P03365-Pro
EAAKEAAKEAAKEAAK	GALV_P21414
GGSEAAKGGG	MMTVB_P03365-Pro

Linker amino acid sequence	RT domain name
SGGSSGGSSGSETPGTSESATPESSGGSSGGSS	MMTVB_P03365-Pro
GSSEAAKGGSS	MMTVB_P03365
GGGSGGGSGGGSGGGSGGGSGGGSGGGSS	HTL3P_Q4U0X6_2mutB
GGGEAAK	MMTVB_P03365-Pro
PAPAPAPAP	MMTVB_P03365-Pro
PAPGSSGGG	MMTVB_P03365
GSSGSSGSSGSSGSS	GALV_P21414
GGSPAP	MMTVB_P03365_WS
GGGSGGGSGGGSGGGSGGGSGGGSGGGSS	MMTVB_P03365-Pro
PAPEAAK	MMTVB_P03365-Pro
PAPGSSGGG	SFV1_P23074-Pro_2mutA
GGGGSEAAK	MMTVB_P03365_2mutB_WS
PAPAPAPAPAP	MMTVB_P03365-Pro
EAAKGGSGSS	MMTVB_P03365-Pro
EAAKEAAKEAAKEAAK	MLVRD_P11227_3mut
PAPAPAPAP	FOAMV_P14350_2mutA
GGGPAPGSS	SFVCP_Q87040_2mut
PAPEAAKGGSS	SFVCP_Q87040_2mut
GGSPAPGGG	MMTVB_P03365-Pro
GGGSGGGSGGGSGGGSGGGSS	MMTVB_P03365
EAAKGGSS	HTL3P_Q4U0X6_2mut
PAPGSSGSS	MMTVB_P03365_WS
GGGSGGGSS	MMTVB_P03365
GGSGSS	FOAMV_P14350
EAAKGGGGSEAAK	SFVCP_Q87040-Pro_2mut
EAAKEAAKEAAKEAAK	MMTVB_P03365-Pro_2mutB
PAPGGGEAAK	SFVCP_Q87040-Pro
GSSGSS	JSRV_P31623_2mutB
EAAKGGGGSS	MMTVB_P03365_2mut_WS
GSSPAPEAAK	MMTVB_P03365-Pro
GGGEAAK	HTL1C_P14078
PAPEAAKGGSS	HTL32_Q0R5R2_2mutB
GGGSSSEAAK	MMTVB_P03365-Pro
PAPGSSGSS	MMTVB_P03365-Pro
EAAKGGGGSS	MMTVB_P03365
GGGSGGGSGGGSGGGSGGGSS	MMTVB_P03365
EAAKGGGGSS	HTL3P_Q4U0X6_2mut
GGGEAAKGGSS	SFVCP_Q87040-Pro
GGGGGSPAP	MMTVB_P03365-Pro_2mutB
GGSGGGEAAK	SFV3L_P27401-Pro

Linker amino acid sequence	RT domain name
PAPGGGGGS	SFV3L_P27401-Pro
EAAAKGGGSEAAK	MMTVB_P03365
PAPEAAKGSS	MMTVB_P03365-Pro
GGSEAAKGGG	MMTVB_P03365-Pro
GGSGSGSGSGSGGS	SMRVH_P03364_2mutB
GGSGSGSGSGSGGS	HTL1L_P0C211_2mut
GGGGGG	WDSV_O92815
GGGGGSGSS	MMTVB_P03365-Pro
GGSEAAKPAP	SFV3L_P27401-Pro_2mut
GGGPAPGSS	MMTVB_P03365_2mut_WS
GGGGGS	MMTVB_P03365_WS
GGSPAPEAAK	MMTVB_P03365
PAPEAAKGSS	HTL1A_P03362
EAAKGGSGSS	MMTVB_P03365_2mut_WS
GGGPAPEAAK	SFV3L_P27401-Pro_2mut
PAPGGGGSS	HTL32_Q0R5R2_2mut
GSSPAPGGG	HTL3P_Q4U0X6_2mut
GGGGSSGGS	BLVAU_P25059_2mut
EAAKGGGGGS	HTL1L_P0C211
GGSEAAKGSS	JSRV_P31623_2mutB
GSSGGG	JSRV_P31623
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EAAKPAP	SFV1_P23074-Pro_2mutA
GGGGSSGGS	MMTVB_P03365_WS
GGSGGS	MMTVB_P03365_WS
EAAKGGGGGS	MMTVB_P03365-Pro
GGGGSGGGSGGGSGGGSGGGSGGGGS	MMTVB_P03365
GGSGSGSGGS	MMTVB_P03365
GGGGGSEAAK	MLVBM_Q7SVK7
GGSGSSPAP	MMTVB_P03365_WS
EAAKEAAKEAAK	JSRV_P31623
PAPEAAKGSS	MMTVB_P03365-Pro
GGSGSSEAAK	FOAMV_P14350
GGGGGSGSS	MMTVB_P03365-Pro_2mut
GGGPAPGGS	MMTVB_P03365
SGSETPGTSESATPES	SFVCP_Q87040_2mut
GSSPAPGGS	SFV1_P23074-Pro_2mutA
GSSSGSSSGSSSGSS	MMTVB_P03365
EAAKGGGPAP	MMTVB_P03365
GSSGGG	MMTVB_P03365_2mut_WS

Linker amino acid sequence	RT domain name
GGGEAAKPAP	MMTVB_P03365
PAPGGSGGG	MMTVB_P03365-Pro
GSSGGSGGG	WDSV_Q92815_2mut
GGSGGG	HTL32_Q0R5R2_2mut
EAAKGGSPAP	HTLV2_P03363_2mut
GGSPAPEAAK	MMTVB_P03365-Pro
GSSGGSEAAK	MMTVB_P03365_2mut
GSAGSAAGSGEF	MMTVB_P03365_WS
PAPGGSGSS	FFV_Q93209
GGSEAAKGGG	MMTVB_P03365
GGSPAPGSS	MMTVB_P03365-Pro
GSSGGSGGG	SFV3L_P27401
PAPEAAKGGG	HTL1A_P03362_2mutB
GGGEAAKPAP	MMTVB_P03365-Pro
GGSEAAK	HTL32_Q0R5R2_2mutB
GGGEAAKGSS	MPMV_P07572
GGGGSEAAK	MMTVB_P03365-Pro
PAPAPAPAPAP	SFVCP_Q87040-Pro_2mutA
PAPAPAPAPAP	HTL1L_P0C211_2mut
GGGSSSGGS	HTL3P_Q4U0X6
PAPGGSEAAK	MMTVB_P03365_2mut_WS
PAPAPAPAPAP	HTL1A_P03362
EAAKPAPGGG	MMTVB_P03365_2mut_WS
GGSEAAK	MMTVB_P03365_2mut_WS
GGGEAAKGSS	SFV1_P23074-Pro_2mutA
GGSPAPGSS	MMTVB_P03365-Pro
GGSEAAKPAP	MLVBM_Q7SVK7
PAPEAAKGGG	MMTVB_P03365_2mut_WS
GSSEAAKPAP	MMTVB_P03365-Pro_2mutB
GGGGSEAAKGGGS	MMTVB_P03365-Pro_2mut
GSSEAAKGGS	MMTVB_P03365-Pro_2mutB
GSSGSSGSSGSSGSS	SRV2_P51517_2mutB
GGGGGSPAP	HTL1L_P0C211_2mut
GGSEAAK	MMTVB_P03365
GSSPAPEAAK	SMRVH_P03364_2mutB
GGGPAPGGS	HTL1C_P14078_2mut
GGSPAPEAAK	MMTVB_P03365_WS
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PAPAPAPAP	HTLV2_P03363_2mut
GSSPAPGGG	MMTVB_P03365

Linker amino acid sequence	RT domain name
GSSGSSGSSGSS	MMTVB_P03365-Pro
GGSEAAKGSS	MMTVB_P03365_WS
GSSGSSGGG	MMTVB_P03365_2mutB
GSSGSSGSSGSSGSSGSS	JSRV_P31623_2mutB
GGSEAAKPAP	MMTVB_P03365-Pro
GSSGGSGGG	HTLV2_P03363_2mut
AEEAAKEAAKEAAKEAAKALEAEAAKEAAKEAAKEAAKEAAKA	WDSV_O92815_2mut
GGSPAPEAAK	MMTVB_P03365
GGGGSEAAK	MMTVB_P03365
GSSGGGEAAK	SFV1_P23074-Pro_2mutA
GGGGSEAAKGGGGS	WDSV_O92815_2mut
GGSGSEAAK	MMTVB_P03365_2mutB_WS
GGSEAAKPAP	MMTVB_P03365_WS
GSSGGGEAAK	SFVCP_Q87040-Pro
GSSGGS	SFVCP_Q87040-Pro_2mut
GGSEAAKPAP	SFVCP_Q87040_2mut
GSSGGSEAAK	SFVCP_Q87040_2mut
GSSPAPEAAK	SRV2_P51517_2mutB
GGSGSGSGSGSGSGSGS	BLVAU_P25059
GSSGSSGSSGSSGSS	HTLV1_P14078_2mut
EAAKGGGSS	MMTVB_P03365_2mutB
GGGEAAKGSS	SFVCP_Q87040-Pro

Example 4: Gene modifying systems comprising Cas-Pol fusions with various human DNA polymerase theta domains enable precise editing in human cells

In this example, exemplary gene modifying polypeptides comprising a Cas domain and a polymerase domain (Cas-Pol fusions), were characterized and their ability to programmably install mutations in genomic DNA in human cells was determined. The gene modifying polypeptides comprised different truncations of an exemplary human polymerase (human DNA Polymerase Theta (Polθ) (Figure 2, Table 23): the gene modifying polypeptide included the polymerase domain, exonuclease domain, and either of two truncated central domains (Polθ_M or Polθ_L) or lacked a central domain (Polθ_4x0q). The gene modifying polypeptides comprised an exemplary Cas domain, SpCas9, comprising an N863A mutation (nCas9). Two different linker sequences were used to connect the Cas domain and Pol domain:

Exemplary linker FL:

DNA sequence:

TCTGGAGGATCTAGCGGAGGATCCTCTGGCAGCGAGACACCAGGAACAAGCGAGTC
AGCAACACCAGAGAGCAGTGGCGGCAGCAGCGGCGGCAGCAGC (SEQ ID NO: 4400)

Amino acid sequence:

5 SGGSSGGSSGSETPGTSESATPESSGGSSGGSS (SEQ ID NO: 4401)

Exemplary linker UL:

DNA sequence:

10 GCCGAAGCCGCCGCCAAGGAGGCCGCCGCTAAGGAGGCTGCTGCCAAGGAAGCTGC
TGCTAAGGCTTTAGAAGCTGAAGCTGCTGCCAAGAAGCTGCCGCCAAGAGGCTG
CCGCAAAGGAGGCCGCTGCCAAGGCT (SEQ ID NO: 4402)

Amino acid sequence:

AEAAAKEAAAKEAAAKEAAAKALEAEAAAKEAAAKEAAAKEAAAKA (SEQ ID NO:
4403)

Six gene modifying polypeptides (Cas-Pol fusions) were tested: (1) nCas9-FL-Polθ_M, (2)
15 nCas9-FL-Polθ_4x0q, (3) nCas9-UL-Polθ_L, (4) nCas9-UL-Polθ_M, (5) nCas9-UL-Polθ_4x0q,
and (6) a benchmark gene modifying polypeptide comprising nCas9 and a reverse transcriptase
domain instead of a polymerase domain.

Table 23: Exemplary Cas-Pol Fusion Gene Modifying Polypeptides

Name	Amino acid sequence	SEQ ID NO
Polθ_L	GASFDLSPGLQRILDKVSSPLENEKLKSMITNFSSLNKNTLNELNEEQEVISNLETKQVQGIFSSNNNEVKS KIEMLENNANHDETSSLLPRKESNIVDDNGLIPPTPIPTSASKLTFFGILETPVNPWKTNVNLQPGESYLF SPSDIKNHDLSPGSRNGFKDNPSIDTSFSLQLSQDGLQLTPASSSESLSIIDVASDQNLFTFIKEWRC KKRFSISLACEKIRSLTSSKTATIGSRFKQASSPQEIPIRDDGFPIKGCDDTLVVGAVCWGGRDAYFYSL QKEQKHSEISASLVPPSLDPSLTCLKDRMWYLQSLRKESDKECSVVIYDFIQSYKILLSCGISLEQSYEDP KVACWLLDPDSQEPTLHSIVTSFLPHLPLLEGMETSSQGIQSLGLNAGSEHSGRYRASVESILIFNSMNQL NSLLQKENLQDVFRKVEMPSSQYCLALLELNGIGFSTAECESQKHIMQAKLDAIETQAYQLAGHSFSFTSS DDIAEVLFLLEKLPPNREMKNQSGSKTLGSTRRGIDNGRKLRLGRQFSTSKDVLNKLKALHPLPGLILEW RRITNAITKVVFPLQREKCLNPFLGMEIYIPVSQSHTATGRITFTPEPNIQNVPRDFEIKMPTLVGESPPSQA VGKGLLPMGRGKYKGFVNPRCQAQMEERAADRGMPFSISMRHAFVFPFGGSILAADYSQLELRILAH LSHRRILQVLTGADVFRSIAAEWKMIPEESVGGDLRQQAQICYGIIYGMGAKSLGEQMGIKENDAAC YIDSFKSRYTGINQFMTETVKNCKRDGFVQTLGRRRYLPGIKDNPNPYRKAHAERQAINTIVQGSAAIDVVI ATVNIQKQLETFHSTFKSHGHREGMLQSDQTGLSRKRKLQGMFCPIRGFFILQLHDELLYEVAEEDVVQ VAQIVKNEMESA VKLSVKLVKVKIGASVWELKDFDV	4404
Polθ_M	GFKDNPSIDTSFSLQLSQDGLQLTPASSSESLSIIDVASDQNLFTFIKEWRCCKRFSISLACEKIRSLT SSKTATIGSRFKQASSPQEIPIRDDGFPIKGCDDTLVVGAVCWGGRDAYFYSLQKEQKHSEISASLVPPS LDPSLTCLKDRMWYLQSLRKESDKECSVVIYDFIQSYKILLSCGISLEQSYEDPKVACWLLDPDSQEPTL HSIVTSFLPHLPLLEGMETSSQGIQSLGLNAGSEHSGRYRASVESILIFNSMNQLNSLLQKENLQDVFRK VEMPSSQYCLALLELNGIGFSTAECESQKHIMQAKLDAIETQAYQLAGHSFSFTSSDDIAEVLFLLEKLPPNR	4405

	EMKNQGSKKTGSTRRGIDNGRKLRLGRQFSTSKDVLNKLKALHPLPGLILEWRRITNAITKVVFPLQREK CLNPFLGMERIYPVQSHTATGRITFTPEPNQNVPRDFEIKMPTLVGESPPSQAVGKGLLPMGRGKYKKG FSVNPQRCAQMEERAADRGMPFSISMRHAFVFPFGGSILAADYSQLELRILAHLSHDDRRIQVLNTGADV FRSIAAEWKMIEPESVGDDLRQQAQKICYGIIYGMGAKSLGEQMGIKENDAACYIDSFKSRYTGINQFMTE TVKNCKRDGFVQTLGRRRYLPGIKDNNPYRKAHAERQAINIVQGSAAIDVKIATVNIQKQLETFHSTFKS HGHREGMLQSDQGTGLSRKRKLQGMFCPIRGFFILQLHDELLYEVAEEDVVQVAQIVKNEMESAVKLSV KLKVKVKGASWGELKDFDV	
Polθ_4x0q	SSSESLSIIDVASDQNLFTFIKEWRCKKRFSISLACEKIRSLTSSKTATIGSRFKQASSPQEIPIRDDGFPI KGCDDTLVVGLAVCWGGRDAYYFSLQKEQKHSEISASLVPPSLDPSLTCLKDRMWYLQSCLRKESDKEC SVVIYDFIQSYKILLSCGISLEQSYEDPKVACWLLDPDSQSEPTLHSIVTSFLPHELPLLEGMETSQGIQSLG LNAGSEHSGRYRASVESILIFNSMNQLNSLLQKENLQDVFVRKVEMPSSQYCLALLELNGIGFSTAECESQK HIMQAKLDAIETQAYQLAGHSFSTSSDDIAEVLFLKLPPNREMKNQGSKKTGSTRRGIDNGRKLRLG RQFSTSKDVLNKLKALHPLPGLILEWRRITNAITKVVFPLQREKCLNPFLGMERIYPVQSHTATGRITFT PNQNVPRDFEIKMPTLVGESPPSQAVGKGLLPMGRGKYKKGFSVNPQRCAQMEERAADRGMPFSIS MRHAFVFPFGGSILAADYSQLELRILAHLSHDDRRIQVLNTGADVFRSIAAEWKMIEPESVGDDLRQQAQK CYGIIYGMGAKSLGEQMGIKENDAACYIDSFKSRYTGINQFMTETVKNCKRDGFVQTLGRRRYLPGIKDN NPYRKAHAERQAINIVQGSAAIDVKIATVNIQKQLETFHSTFKSHGHREGMLQSDQGTGLSRKRKLQGMF CPIRGFFILQLHDELLYEVAEEDVVQVAQIVKNEMESAVKLSVKLKVKVKGASWGELKDFDV	4406
Polθ_4x0q _GS	SSSESLSIIDVASDQNLFTFIKEWRCKKRFSISLACEKIRSGDDTLVVGLAVCWGGRDAYYFSLGGS GGLDPSLTCLKDRMWYLQSCLRKESDKECSVVIYDFIQSYKILLSCGISLEQSYEDPKVACWLLDPDSQ PTLHSIVTSFLPHELPLLEGMETSQGIQSLGLNAGSEHSGRYRASVESILIFNSMNQLNSLLQKENLQDVF RKVEMPSSQYCLALLELNGIGFSTAECESQKHIMQAKLDAIETQAYQLAGHSFSTSSDDIAEVLFLKL PPGSGGQFSTSKDVLNKLKALHPLPGLILEWRRITNAITKVVFPLQREKCLNPFLGMERIYPVQSHTATG RITFTPEPNQNVPRDFEIKMGSGGMPFSISMRHAFVFPFGGSILAADYSQLELRILAHLSHDDRRIQVLNT GADVFRSIAAEWKMIEPESVGDDLRQQAQKICYGIIYGMGAKSLGEQMGIKENDAACYIDSFKSRYTG QFMTETVKNCKRDGFVQTLGRRRYLPGIKDNNPYRKAHAERQAINIVQGSAAIDVKIATVNIQKQLETFH STFKSHGHREGMLQSDGSGGCPIRGGFFILQLHDELLYEVAEEDVVQVAQIVKNEMESAVKLSVKLK VKVKGASWGELKDFDV	4407
nCas9	DKKYSIGLDIGTNSVGWAVITDEYKVPSSKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRY TRRNRIQCYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKKHERHPFGNIVDEVAYHEKYPTIYHLRKKLVD STDKADRLIYLAHAMIKFRGHFLIEGDLNPDNSDVKLFQLVQTYNQLFEENPINASGVDAKAILSARLS KSRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDQYDDDLNLLAQIGDQYAD LFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG YIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYFPL KDNREKIEKILTRIPYVYVGLPARGNSRFAWMTRKSEETITPWNFEVVDKGASQSFIERMTNFDKNLPN EKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECF DSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEENEDILEDIVLTTLFEDREMIERLKYAHLFDDKVM KQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDFANRNFMLIHDDSLTFKEDIQKAQVSGQGD SLHEHIANLAGSPAIIKGIQTQVVMDELVKVMGRHKPENIVIAMARENQTTQKGQKNSRERMRIIEGIG ELGSQILKEHPVENTQLQNEKLYLYLQNGRDMYVDQELDNRLSDYVDVHIVPQSFLKDDSIDNKVLT DKARGKSDNVPSSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRIQTH VAQILD SRMNTKYDENDKLIREVKVITLKSLSVDFRKFQFYKVINNYHHAHDAYLNAVVG TALIKKYP KLESEFYVYDYVDVRKMIKSEQIEGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVV DKGRDFATVRKVL SMPQVNIKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKGFGFDSPTVAYS VVAKEVGKSKLKS VKELLGITIMERSSEFKNPIDFLEAKGYKEVKKDLIIPKYSLEFENGRKRLAS AGELQKGNELALPSKYVNFYLA SHYEKLKGPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVLADANL DKVLSAYNKHDKPIREQAENIHLFTLTNLGAPAAFKYFDTTIDRKYRTSTKEVLDTLIHQSTIGLYETRID LSQLGGD	4408

- Test template nucleic acids were constructed coding for a direct a single base pair substitution which, when introduced as an edit by the exemplary gene modifying polypeptides, act to convert an exogenous BFP marker to GFP (as described herein). Template nucleic acids comprised (i) a gRNA spacer sequence for guiding the gene modifying polypeptide to the target region, e.g., a sequence complementary to a 20-nucleotide sequence in BFP locus; (ii) a primer-binding sequence (PBS) capable of complementary base pairing with a single strand of the nicked DNA for target-primed polymerization; (iii) a heterologous object sequence providing a template for polymerization that further comprises the intended final target sequence; and (iv) a gRNA

scaffold sequence to associate with the Cas9 domain of the gene modifying polypeptide. The exemplary template nucleic acids were arranged, from 5' to 3' in the following order: (i)-(iv)-(iii)-(ii). Template compositions are described in Table 24 (RNACS-4239-DD, and RNACS-4241-DR), where (ii) and (iii) may each be included as either RNA or DNA nucleotides.

5 **Table 24: Template nucleic acids used in Example 1**

Name	Description	Spacer (i)	Scaffold (iv)	Pol template (iii)	PBS (ii)	Full Template Molecule
Template DD	BFP_g4_11RT_13PBS_DD	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrU (SEQ ID NO: 4409)	rGrUrUrUrUrArGrArGrCrUrArGrArArArArUrArGrCrArArGrUrUrArArArArArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArCrUrUrGrArArArArGrUrGrGrCrCrGrGrGrU (SEQ ID NO: 4411)	CCTGACGTACG (SEQ ID NO: 4413)	GCGTGCAGTG*mC*mU*mU (SEQ ID NO: 4415)	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrUrGrUrUrUrUrArGrArGrCrUrArGrArArArGrArArGrUrUrUrArArArArGrGrCrUrArGrUrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrCrGrArGrUrCrGrGrGrCCCTGACGTACGCGCTGCAGTG*mC*mU*mU (SEQ ID NO: 4417)
Template DR	BFP_g4_11RT_13PBS_DR	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrU (SEQ ID NO: 4410)	rGrUrUrUrUrArGrArGrCrUrArGrArArArArUrArGrCrArArGrUrUrArArArArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArCrUrUrGrArArArArGrUrGrGrCrCrGrGrGrU (SEQ ID NO: 4412)	CCTGACGTACG (SEQ ID NO: 4414)	rGrCrGrUrGrCrArGrUrGr*mC*mU*mU (SEQ ID NO: 4416)	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrUrGrUrUrUrUrArGrArGrCrUrArGrArArArGrGrCrUrArGrUrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrCrGrArGrUrCrGrGrGrCCCTGACGTACGGrGrGrUrGrCrArGrUrG*mC*mU*mU (SEQ ID NO: 4418)
Template RR	BFP_g4_11RT_13PBS_RR	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrU (SEQ ID NO: 4419)	rGrUrUrUrUrArGrArGrCrUrArGrArArArArUrArGrCrArArGrUrUrArArArArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrC (SEQ ID NO: 4420)	rCrCrUrGrArCrGrUrArCrG (SEQ ID NO: 4421)	rGrCrGrUrGrCrArGrUrGr*mC*mU*mU (SEQ ID NO: 4422)	mG*mC*mC*rGrArArGrCrArCrUrGrCrArCrGrCrGrUrGrUrUrUrUrArGrArGrCrUrArGrArArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArGrUrGrGrCrCrGrArGrUrCrGrGrGrCCCTGACGTACGGrGrGrUrGrCrArGrUrG*mC*mU*mU (SEQ ID NO: 4423)

RNA, expressed as 'r_', for example rA, rU; DNA, expressed as 'd_', for example A, T; 2'-O-methyl RNA bases are expressed as 'm_'; Phosphorothioated 2'-O-methyl RNA bases are entered as 'm_*'.

Experiments were performed to determine the genome editing capacity of the Cas-Pol fusion gene modifying polypeptides.

U2OS or HEK293T cells were infected with lentiviral vectors encoding an exemplary gene modifying polypeptide and containing an antibiotic (Puromycin) resistance cassette. The

cells stably expressing the gene modifying polypeptide were selected by culturing the cells in puromycin-supplemented media. 250,000 cells/well (96-well plate) were nucleofected with 2.5 uM and 5 uM of the chemically synthesized exemplary template nucleic acid molecules. Cells were subjected to Fluorescence-activated Cell Sorting (FACS, flow) on day 3 post-nucleofection.

5 The frequency of intended editing (conversion of BFP positive cells into GFP positive cells) was determined as the percentage of GFP positive cells relative to the percentage of BFP positive cells (FIGs. 3A and 3B).

The results show that gene modifying polypeptides nCas9-UL- Polθ _L, nCas9-UL- Polθ _M, nCas9-UL- Polθ _4x0q, nCas9-FL- Polθ _M, and nCas9-FL- Polθ _4x0q exhibited editing

10 activity, e.g., up to ~75% editing efficiency in HEK293 cells. The gene modifying polypeptides showed high editing activity (comparable to or higher than the benchmark gene modifying polypeptide containing a control reverse transcriptase) with template nucleic acid comprising a DNA-encoded heterologous object sequence and an RNA primer binding sequence, denoted as “DR” (FIG. 3A-B). The gene modifying polypeptides also exhibited editing activity with

15 template nucleic acid comprising both DNA heterologous object sequence and DNA primer binding sequence, denoted as “DD”, although less than when an RNA primer binding sequence was used (FIG. 3A-B). The gene modifying polypeptides showed no editing activity with template nucleic acids comprising RNA heterologous object sequence and RNA primer binding sequences, denoted as “RR” (FIG.3A-B). The levels of editing were comparable or higher than

20 the benchmark gene modifying polypeptide, demonstrating that gene modifying polypeptides using polymerase domains are useful alternatives to gene modifying polypeptides using reverse transcriptase domains. The results further demonstrate that a variety of truncations of Polθ can be used as polymerase domains, as the data shows gene modifying polypeptides comprising any truncations tested (including complete truncation of the central domain) had editing activity.

25

It should be understood that for all numerical bounds describing some parameter in this application, such as “about,” “at least,” “less than,” and “more than,” the description also necessarily encompasses any range bounded by the recited values. Accordingly, for example, the description “at least 1, 2, 3, 4, or 5” also describes, inter alia, the ranges 1-2, 1-3, 1-4, 1-5, 2-

30 3, 2-4, 2-5, 3-4, 3-5, and 4-5, et cetera.

For all patents, applications, or other reference cited herein, such as non-patent literature and reference sequence information, it should be understood that they are incorporated by reference in their entirety for all purposes as well as for the proposition that is recited. Where any conflict exists between a document incorporated by reference and the present application, 5 this application will control. All information associated with reference gene sequences disclosed in this application, such as GeneIDs or accession numbers (typically referencing NCBI accession numbers), including, for example, genomic loci, genomic sequences, functional annotations, allelic variants, and reference mRNA (including, e.g., exon boundaries or response elements) and protein sequences (such as conserved domain structures), as well as chemical references (e.g., 10 PubChem compound, PubChem substance, or PubChem Bioassay entries, including the annotations therein, such as structures and assays, et cetera), are hereby incorporated by reference in their entirety.

Headings used in this application are for convenience only and do not affect the interpretation of this application.

15

CLAIMS

What is claimed is:

1. A gene modifying polypeptide comprising:
a Cas domain (e.g., a Cas nickase domain, e.g., a Cas9 nickase domain);
a polymerase (Pol) domain of Table 1 or Table 23, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto, wherein the Pol domain is C-terminal of the Cas domain; and
a linker disposed between the Pol domain and the Cas domain.
2. The gene modifying polypeptide of claim 1, wherein the linker has a sequence from Table 6, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, or 99% identity thereto.
3. The gene modifying polypeptide of claim 1 or 2, wherein the Pol domain has a sequence with at least 90% identity to the Pol domain of Table 1 or 23.
4. The gene modifying polypeptide of any of the preceding claims, wherein the Pol domain has a sequence with at least 95% identity to the Pol domain of Table 1 or 23.
5. The gene modifying polypeptide of any of the preceding claims, wherein the Pol domain has a sequence with at least 98% identity to the Pol domain of Table 1 or 23.
6. The gene modifying polypeptide of any of the preceding claims, wherein the Pol domain has a sequence with at least 99% identity to the Pol domain of Table 1 or 23.
7. The gene modifying polypeptide of any of the preceding claims, wherein the Pol domain has a sequence with 100% identity to the Pol domain of Table 1 or 23.
8. The gene modifying polypeptide of any of the preceding claims, wherein the linker has a sequence with at least 90% identity to the linker sequence from Table 6.

9. The gene modifying polypeptide of any of the preceding claims, wherein the linker has a sequence with at least 95% identity to the linker sequence from Table 6.
10. The gene modifying polypeptide of any of the preceding claims, wherein the linker has a sequence with at least 97% identity to the linker sequence from Table 6.
11. The gene modifying polypeptide of any of the preceding claims, wherein the linker has a sequence with 100% identity to the linker sequence from Table 6.
12. The gene modifying polypeptide of any of the preceding claims, wherein the Cas domain comprises a sequence of Table 4, or a sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identity thereto.
13. The gene modifying polypeptide of any of the preceding claims, wherein the Cas domain is a Cas nickase domain.
14. The gene modifying polypeptide of any of the preceding claims, wherein the Cas domain is a Cas9 nickase domain.
15. The gene modifying polypeptide of any of the preceding claims, wherein the Cas domain comprises an N863A mutation.
16. The gene modifying polypeptide of any of the preceding claims, which comprises an NLS, e.g., wherein the gene modifying polypeptide comprises two NLSs.
17. The gene modifying polypeptide of any of the preceding claims, which comprises an NLS N-terminal of the Cas9 domain.
18. The gene modifying polypeptide of any of the preceding claims, which comprises an NLS C-terminal of the Pol domain.

19. The gene modifying polypeptide of any of the preceding claims, which comprises a first NLS which is N-terminal of the Cas9 domain and a second NLS which is C-terminal of the Pol domain.
20. A nucleic acid (e.g., DNA or RNA, e.g., mRNA) encoding the gene modifying polypeptide of any of the preceding claims.
21. A cell comprising the gene modifying polypeptide of any of claims 1-19 or the nucleic acid of claim 20.
22. A system comprising:
 - i) the gene modifying polypeptide of any of claims 1-19, and
 - ii) a template nucleic acid (e.g., a template RNA) that comprises:
 - a) a gRNA spacer that is complementary to a portion a target nucleic acid sequence;
 - b) a gRNA scaffold that binds the Cas domain of the gene modifying polypeptide;
 - c) a heterologous object sequence; and
 - d) a primer binding site sequence (PBS sequence).
23. The system of claim 22, wherein the template nucleic acid comprises RNA.
24. The system of claim 22 or 23, wherein the template nucleic acid comprises DNA.
25. The system of claim 22, wherein the template nucleic acid comprises DNA and RNA.
26. The system of any of claims 22-25, wherein the template nucleic acid comprises a nucleic acid sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

27. The system of claim 26, wherein the gRNA spacer of the template nucleic acid comprises a spacer sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
28. The system of claim 26 or 27, wherein the gRNA scaffold of the template nucleic acid comprises a scaffold sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
29. The system of any of claims 26-28, wherein the PBS sequence of the template nucleic acid comprises a PBS sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
30. The system of claim 26, wherein the template nucleic acid sequence comprises one or more (e.g., 1, 2, or all 3) of the following, e.g., in 5' to 3' order:
- (i) a spacer sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto;
 - (ii) a scaffold sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and/or
 - (iii) a PBS sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
31. The system of any of claims 26-30, wherein the template nucleic acid comprises a full template molecule sequence as listed in Table 24, or a nucleic acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
32. The system of any of claims 22-31, wherein the gRNA spacer and the gRNA scaffold comprise RNA.
33. The system of any of claims 22-32, wherein the heterologous object sequence comprises DNA and PBS sequence comprise RNA.

34. The system of any of claims 22-32, wherein the heterologous object sequence and PBS sequence comprise DNA.
35. The system of any of claims 22-34, wherein the gene modifying polypeptide comprises an amino acid sequence as listed in Table 23, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
36. The system of any of claims 22-35, wherein the gene modifying polypeptide comprises an amino acid sequence of any one of nCas9-UL- Polθ_L, nCas9-UL- Polθ_M, nCas9-UL- Polθ_4x0q, nCas9-FL- Polθ_M, or nCas9-FL- Polθ_4x0q, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
37. The system of any of claims 22-36, wherein the gene modifying polypeptide comprises a Cas domain amino acid sequence of a gene modifying polypeptide as listed in Table 23, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
38. The system of any of claims 22-37, wherein the gene modifying polypeptide comprises a Cas domain amino acid sequence of any one of nCas9-UL- Polθ_L, nCas9-UL- Polθ_M, nCas9-UL- Polθ_4x0q, nCas9-FL- Polθ_M, or nCas9-FL- Polθ_4x0q, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
39. The system of any of claims 22-38, wherein the gene modifying polypeptide comprises a Pol domain amino acid sequence of a gene modifying polypeptide as listed in Table 23, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.
40. The system of any of claims 22-39, wherein the gene modifying polypeptide comprises a Pol domain amino acid sequence of any one of nCas9-UL- Polθ_L, nCas9-UL- Polθ_M, nCas9-UL- Polθ_4x0q, nCas9-FL- Polθ_M, or nCas9-FL- Polθ_4x0q, or an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

41. The system of any of claims 22-40, wherein the gene modifying polypeptide comprises a Cas domain amino acid sequence and a Pol domain amino acid sequence of a gene modifying polypeptide as listed in Table 23, or amino acid sequences having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

42. The system of any of claims 22-41, wherein the gene modifying polypeptide comprises a Cas domain amino acid sequence and a Pol domain amino acid sequence of any one of nCas9-UL-Polθ_L, nCas9-UL-Polθ_M, nCas9-UL-Polθ_4x0q, nCas9-FL-Polθ_M, or nCas9-FL-Polθ_4x0q, or amino acid sequences having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

43. A method for modifying a target nucleic acid in a cell (e.g., a human cell), the method comprising contacting the cell with the system of any of claims 22-42, or nucleic acid encoding the same, thereby modifying the target nucleic acid.

44. A method for treating a subject having a disease or condition associated with a genetic defect, the method comprising:
administering to the subject a system, polypeptide, template RNA or DNA encoding the same of any of the preceding claims, thereby treating the subject having a disease or condition associated with a genetic defect.

45. The method of claim 44, wherein the disease or condition associated with a genetic defect is an indication listed in any of Tables 12-15 and/or wherein the genetic defect is a defect in a gene listed in any of Tables 12-15.

46. The method of claim 44 or 45, wherein the subject is a human patient.