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(19) **United States**(12) **Patent Application Publication**
Zhang et al.(10) **Pub. No.: US 2015/0232882 A1**(43) **Pub. Date: Aug. 20, 2015**(54) **ENGINEERING OF SYSTEMS, METHODS
AND OPTIMIZED GUIDE COMPOSITIONS
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Cambridge, MA (US)(72) Inventors: **Feng Zhang**, Cambridge, MA (US); **Le
Cong**, Cambridge, MA (US)(21) Appl. No.: **14/703,511**(22) Filed: **May 4, 2015****Related U.S. Application Data**(63) Continuation of application No. PCT/US2013/
074819, filed on Dec. 12, 2013.(60) Provisional application No. 61/748,427, filed on Jan.
2, 2013, provisional application No. 61/758,468, filed
on Jan. 30, 2013, provisional application No. 61/769,
046, filed on Feb. 25, 2013, provisional application
No. 61/791,409, filed on Mar. 15, 2013, provisional
application No. 61/802,174, filed on Mar. 15, 2013,
provisional application No. 61/806,375, filed on Mar.28, 2013, provisional application No. 61/814,263,
filed on Apr. 20, 2013, provisional application No.
61/819,803, filed on May 6, 2013, provisional appli-
cation No. 61/828,130, filed on May 28, 2013, provi-
sional application No. 61/835,931, filed on Jun. 17,
2013, provisional application No. 61/836,127, filed on
Jun. 17, 2013, provisional application No. 61/736,527,
filed on Dec. 12, 2012.**Publication Classification**(51) **Int. Cl.**
C12N 15/90 (2006.01)
C12N 15/85 (2006.01)(52) **U.S. Cl.**
CPC **C12N 15/907** (2013.01); **C12N 15/85**
(2013.01); **C12N 2800/10** (2013.01); **C12N**
2810/50 (2013.01)(57) **ABSTRACT**

The invention provides for systems, methods, and composi-
tions for manipulation of sequences and/or activities of target
sequences. Provided are vectors and vector systems, some of
which encode one or more components of a CRISPR com-
plex, as well as methods for the design and use of such
vectors. Also provided are methods of directing CRISPR
complex formation in eukaryotic cells and methods for
selecting specific cells by introducing precise mutations uti-
lizing the CRISPR-Cas system.

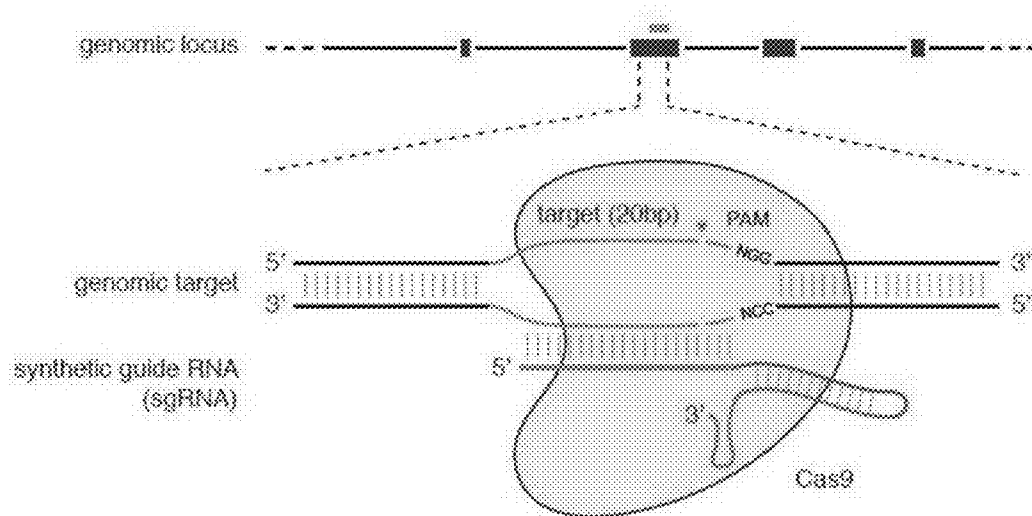


FIG. 1

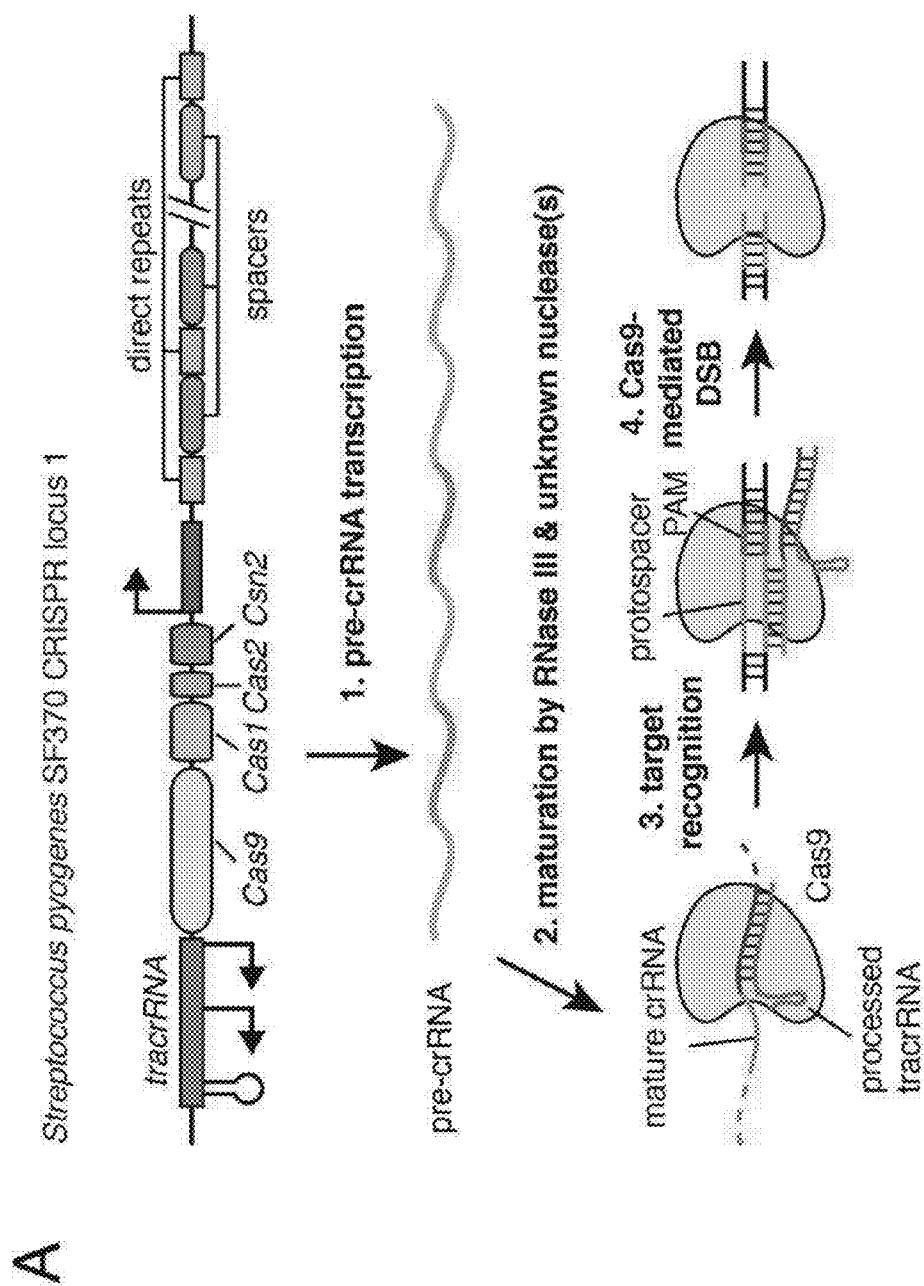


FIG. 2A

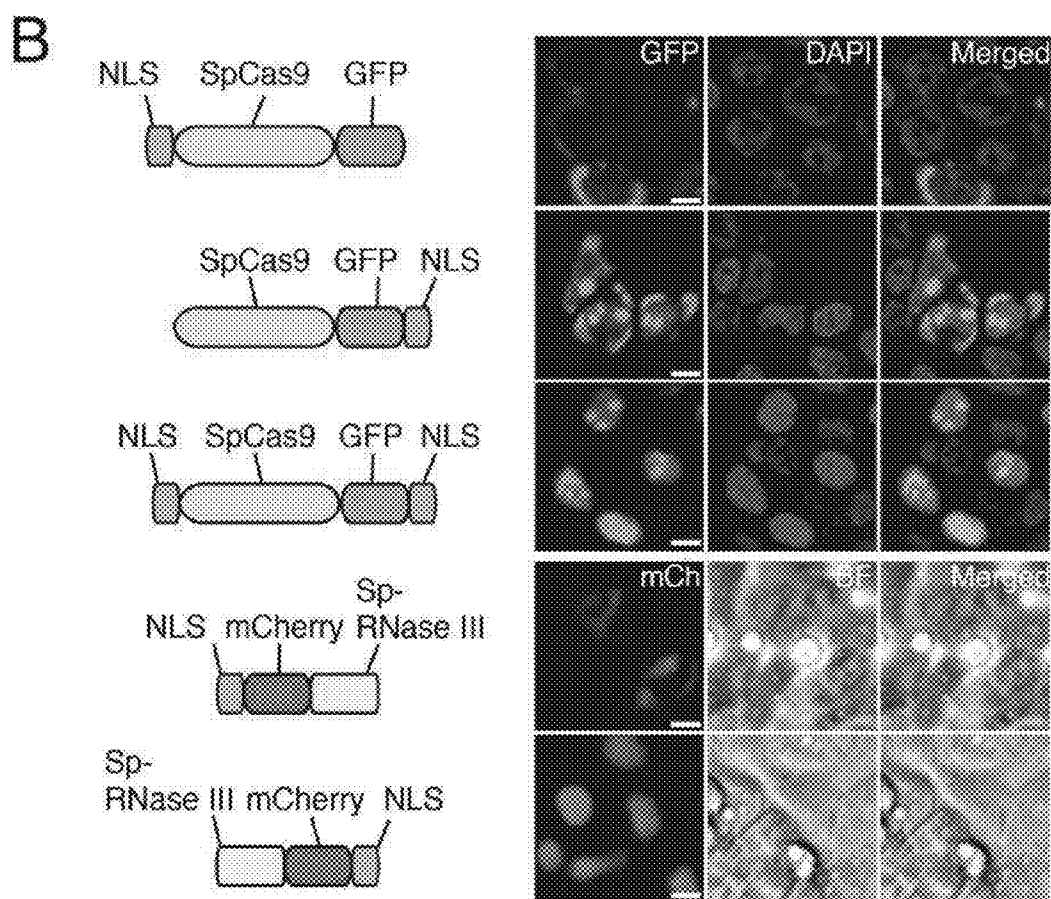


FIG. 2B

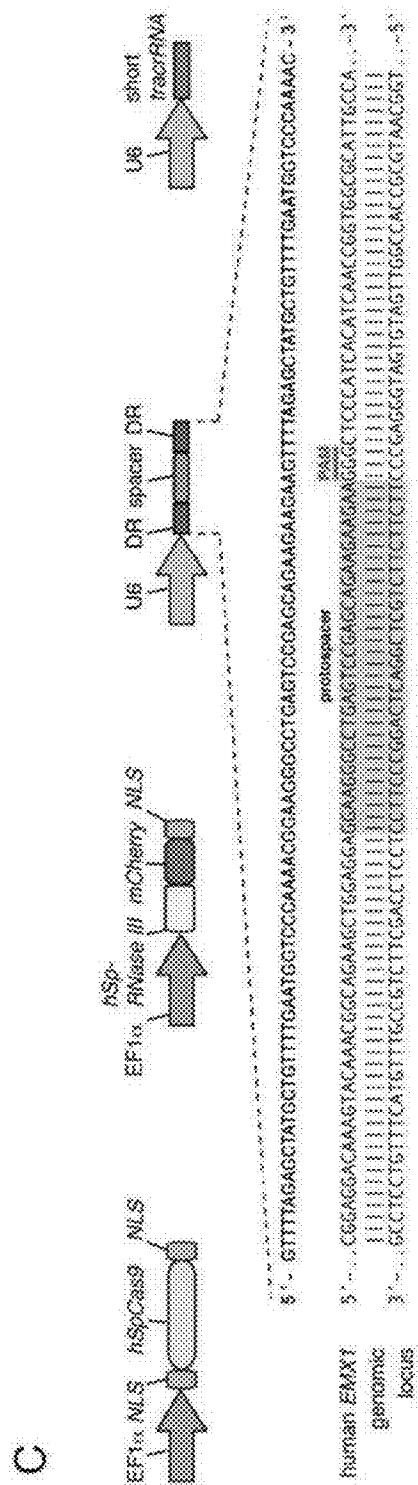


FIG. 2C

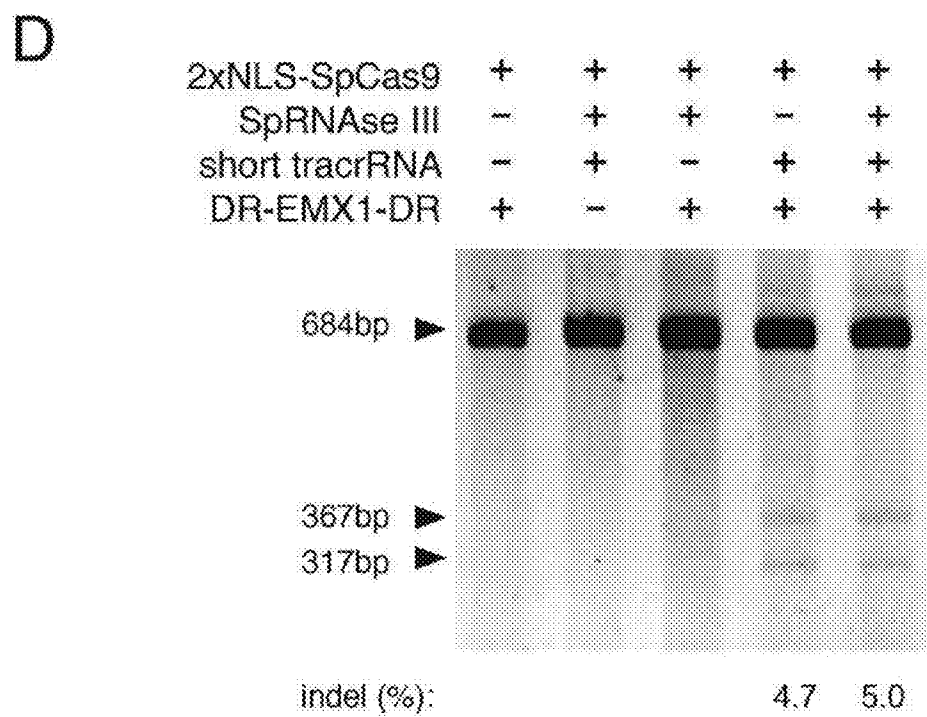


FIG. 2D

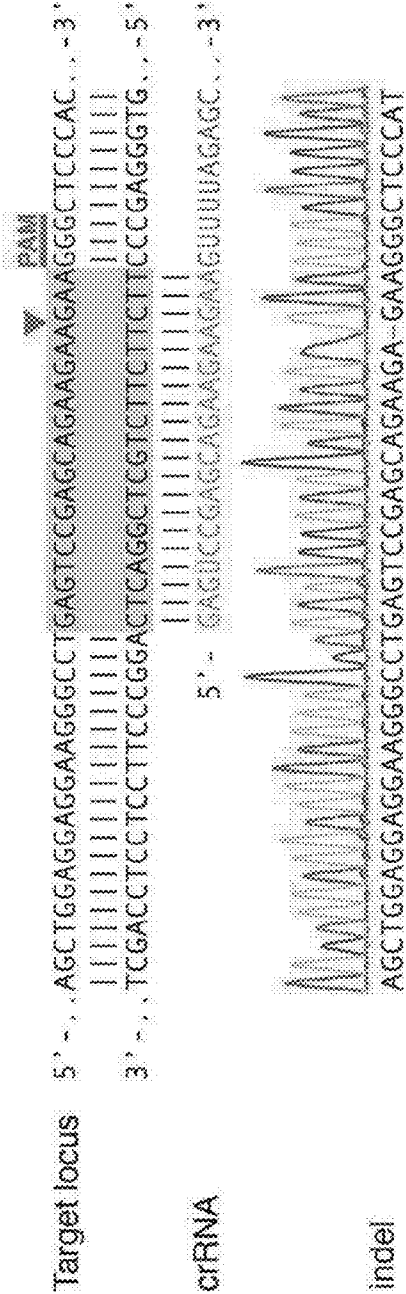
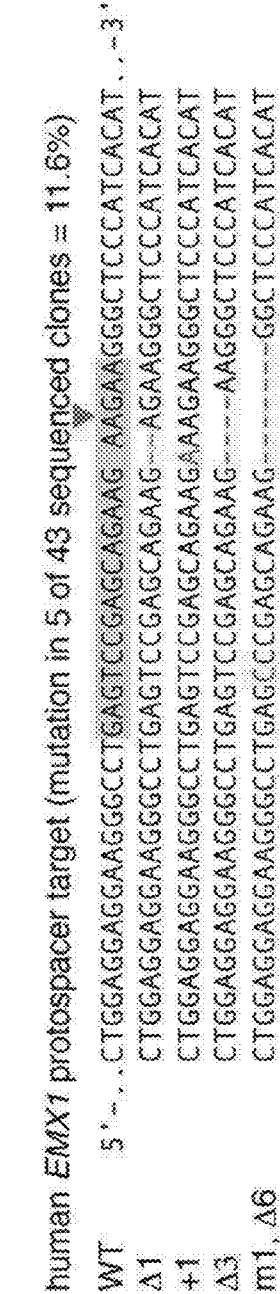


FIG. 2E-F

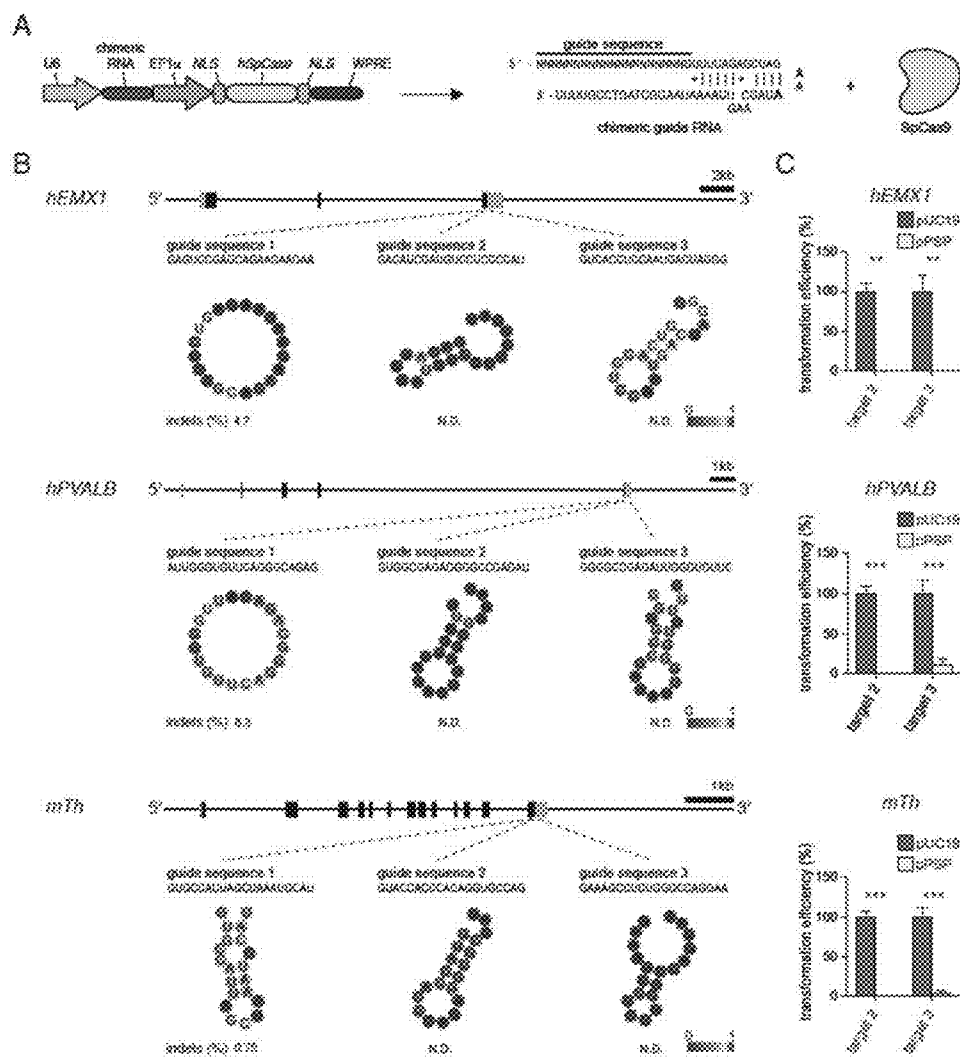


FIG. 3A-C

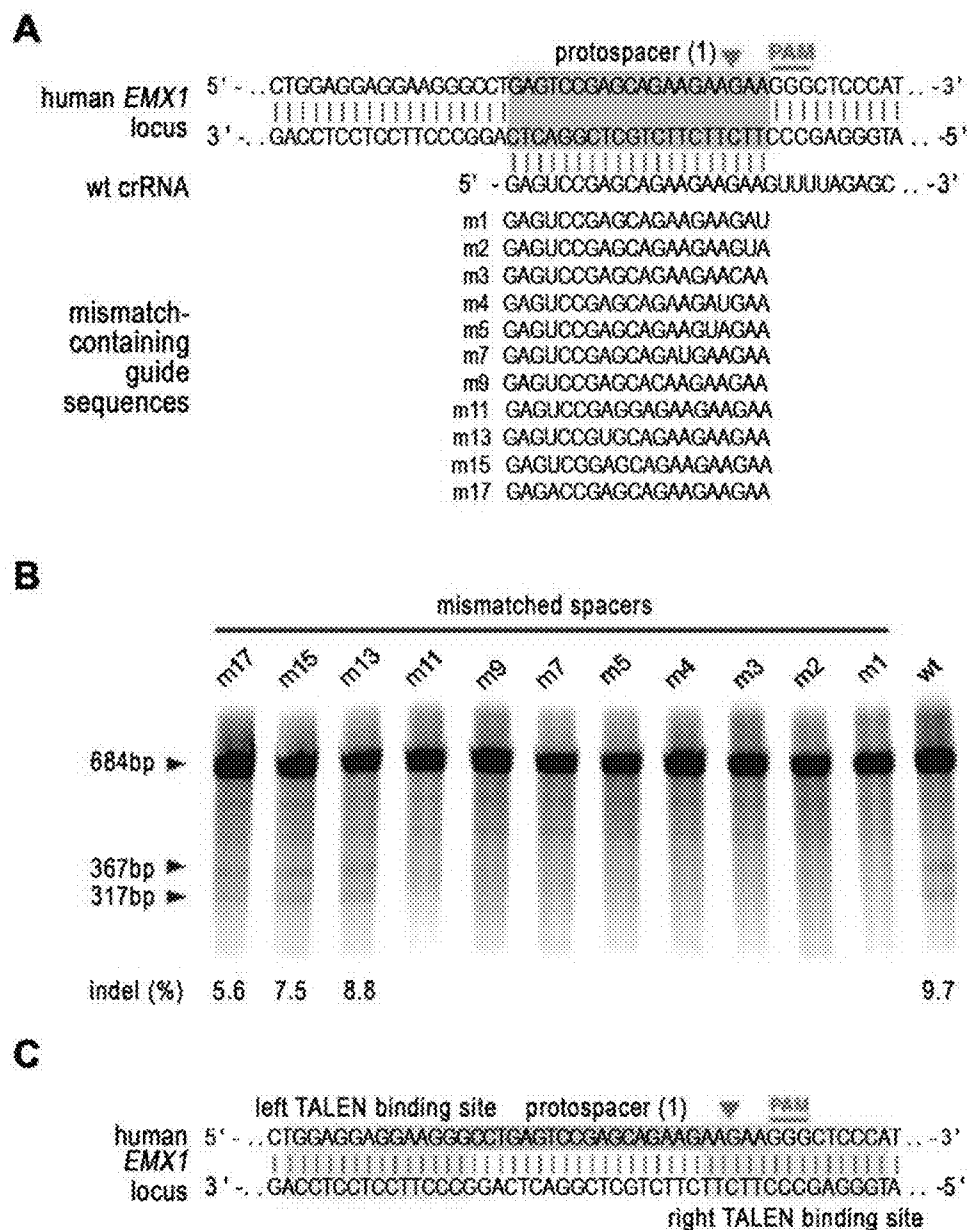


FIG. 4A-C

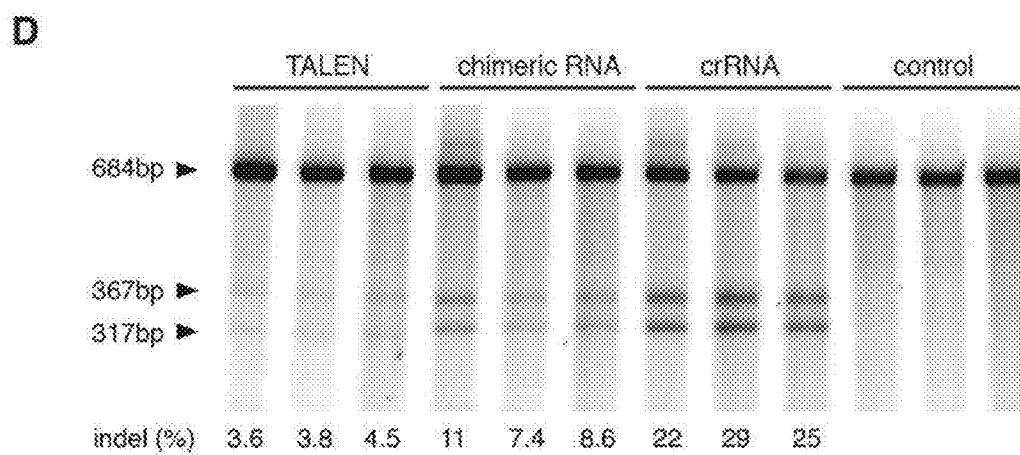
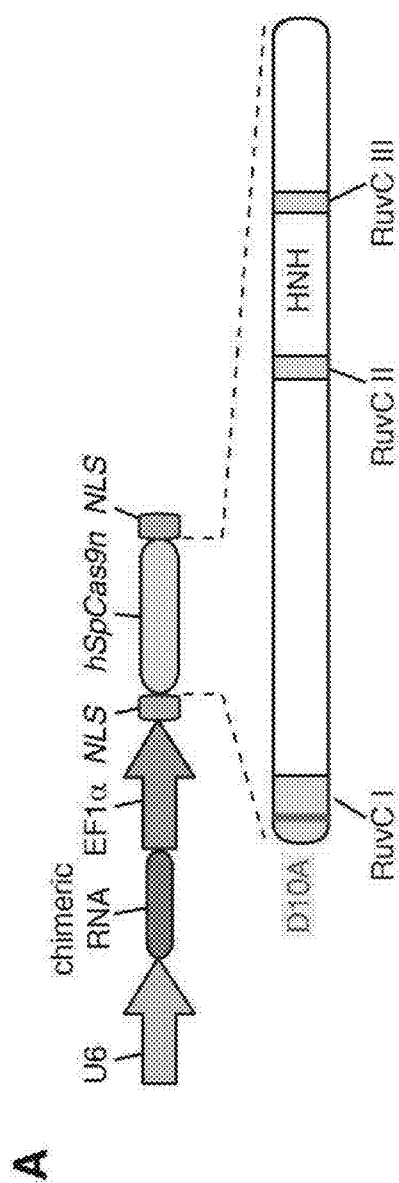
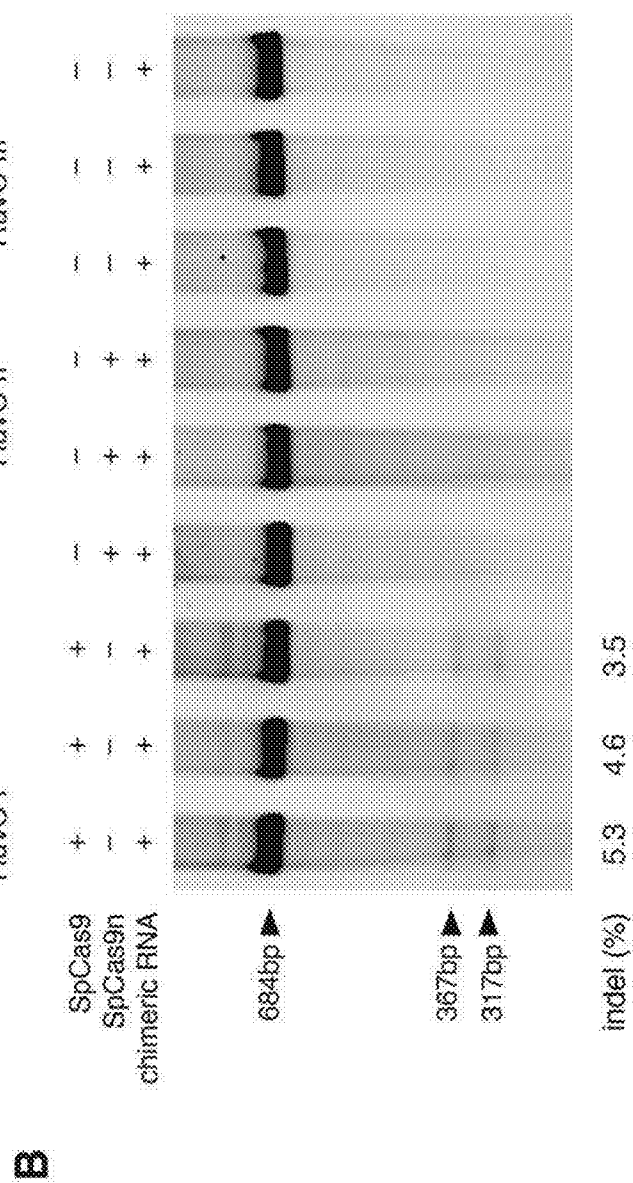


FIG. 4D



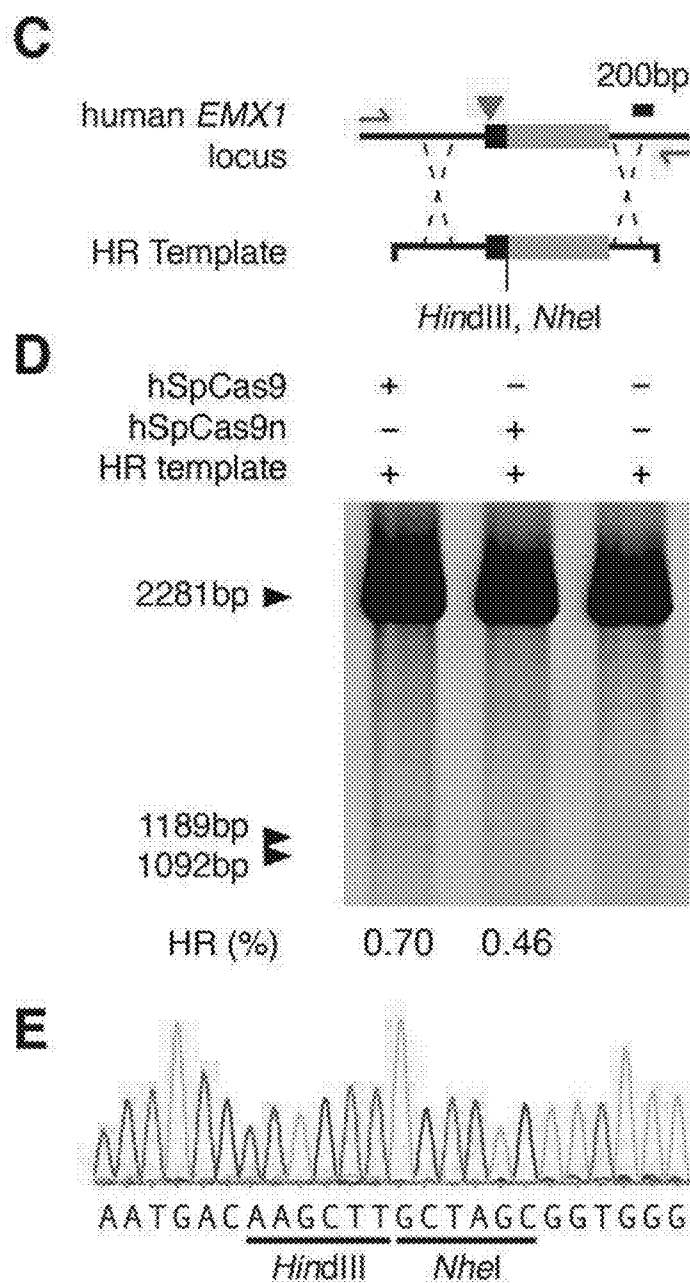


FIG. 5C-E

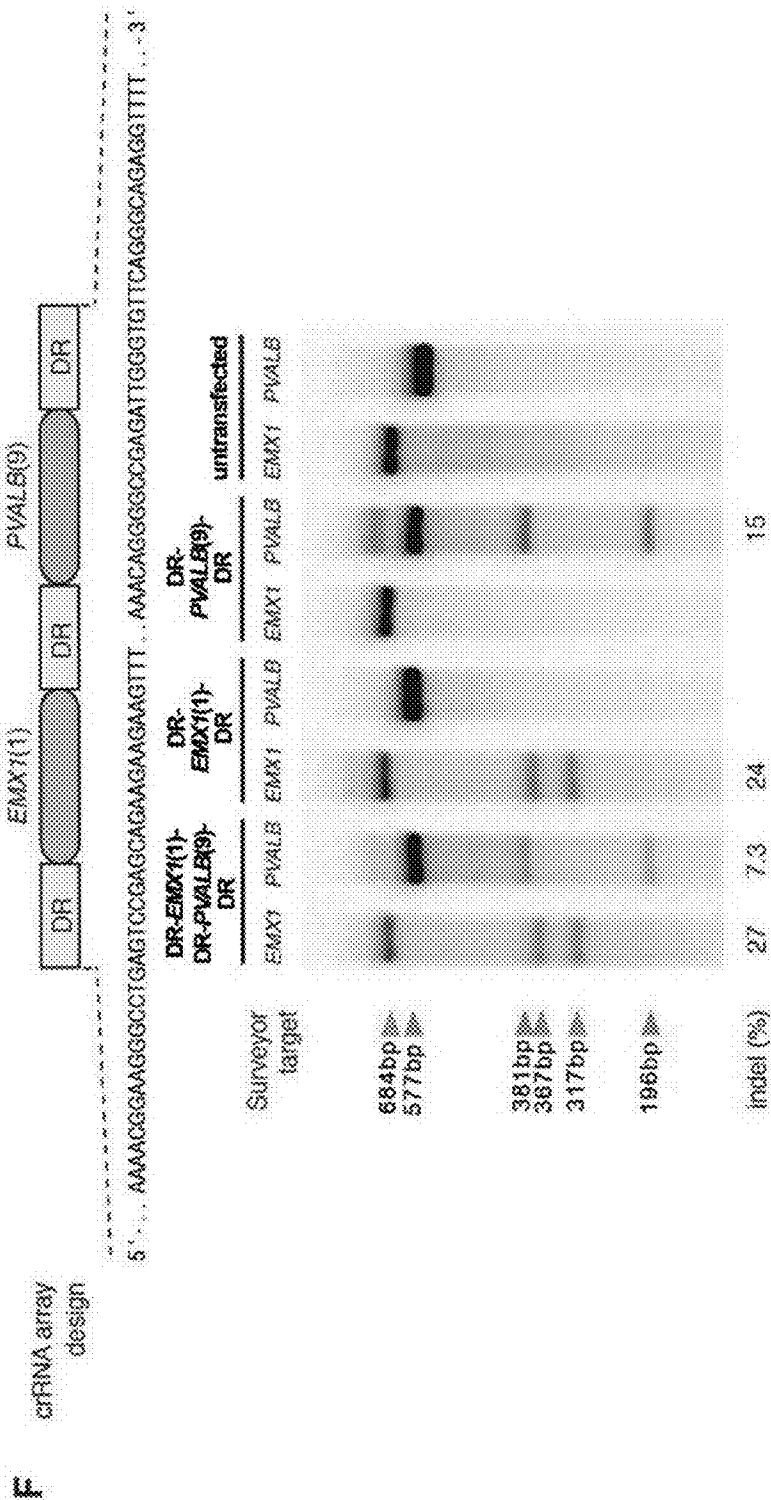


FIG. 5F

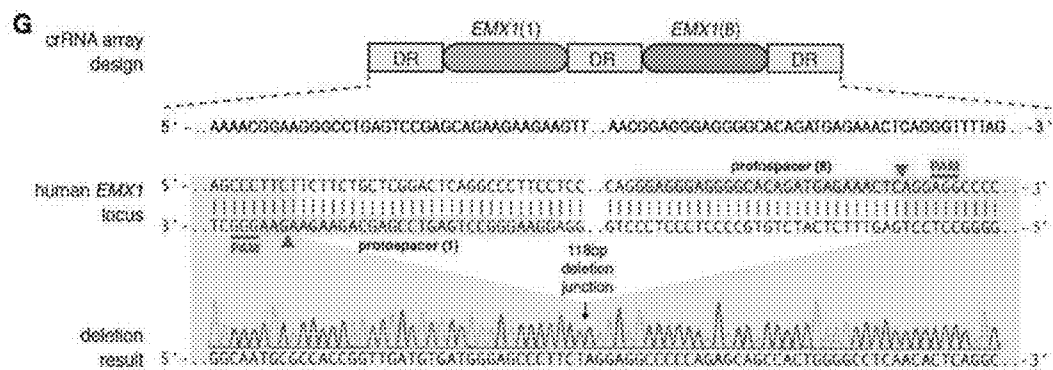
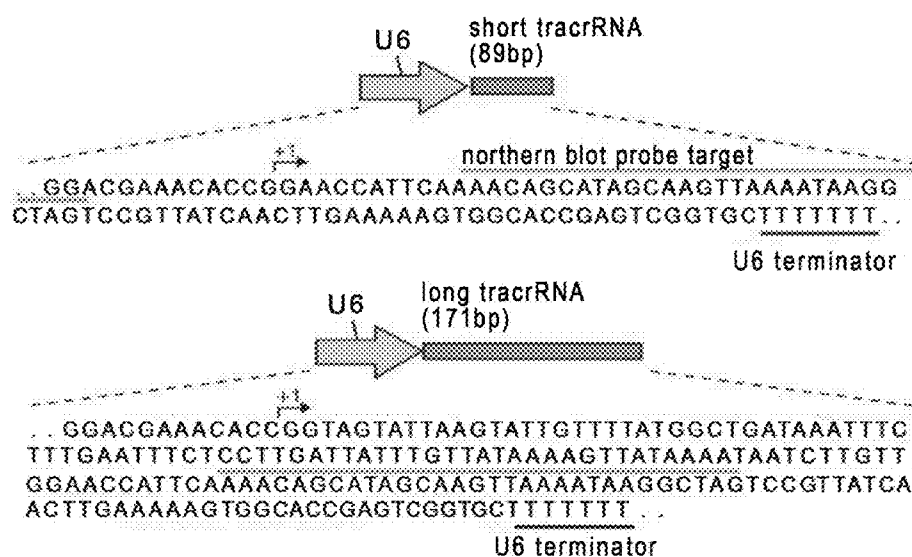


FIG. 5G

A



B

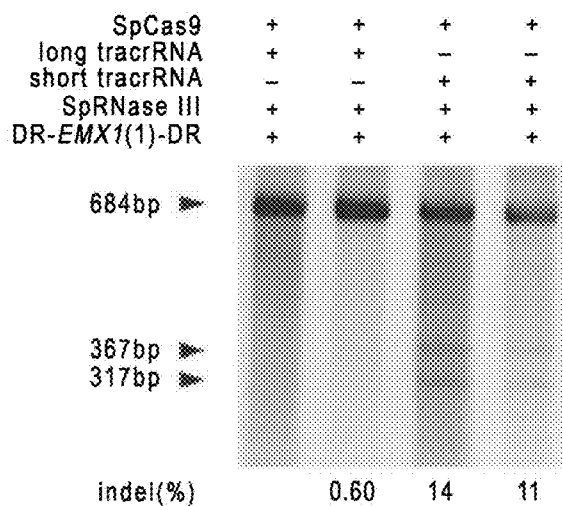


FIG. 6A-B

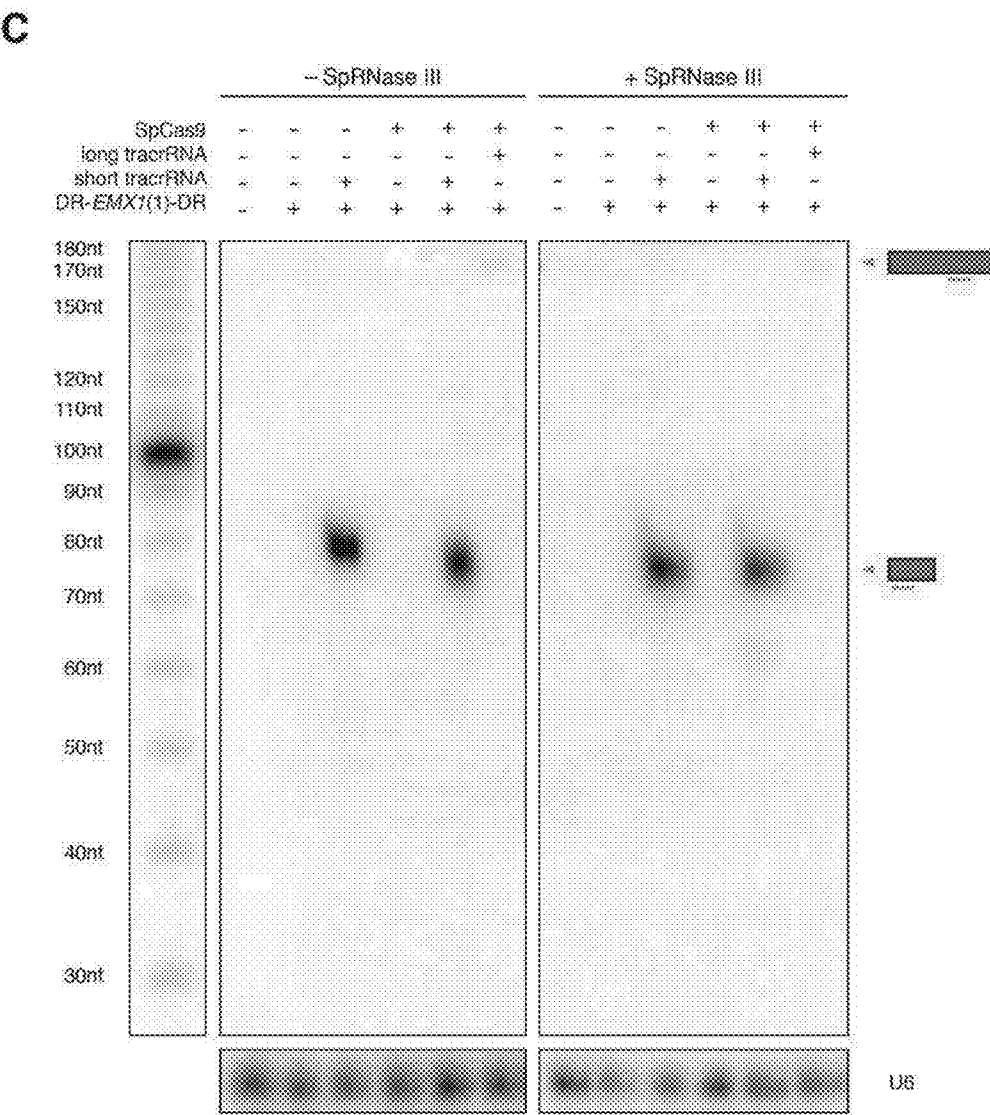
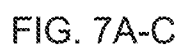


FIG. 6C



D

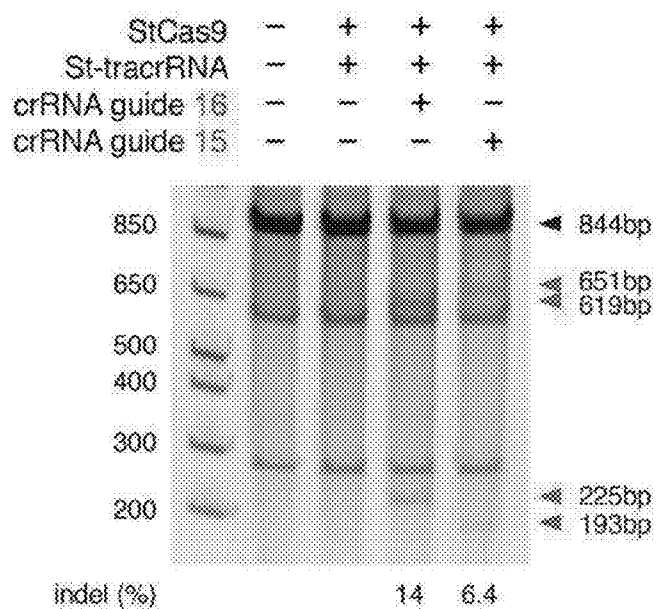
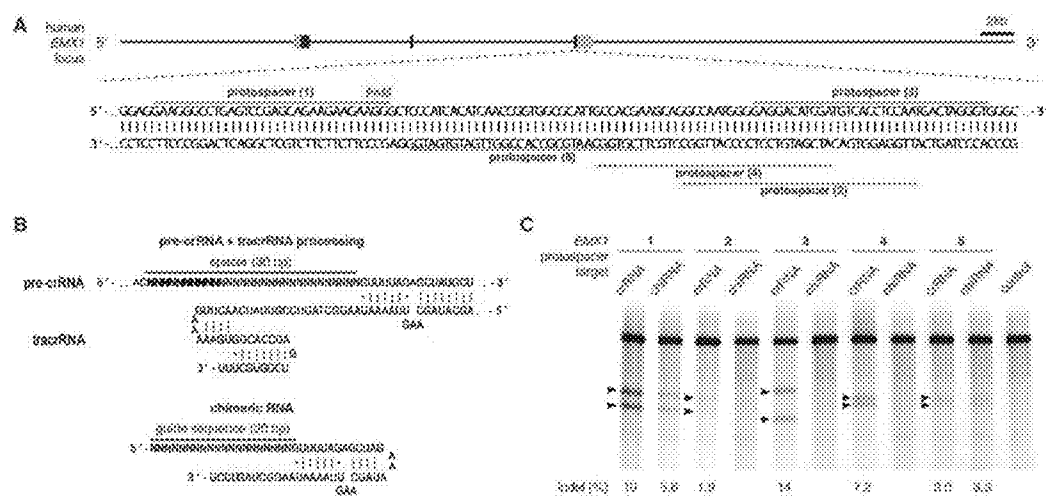


FIG. 7D



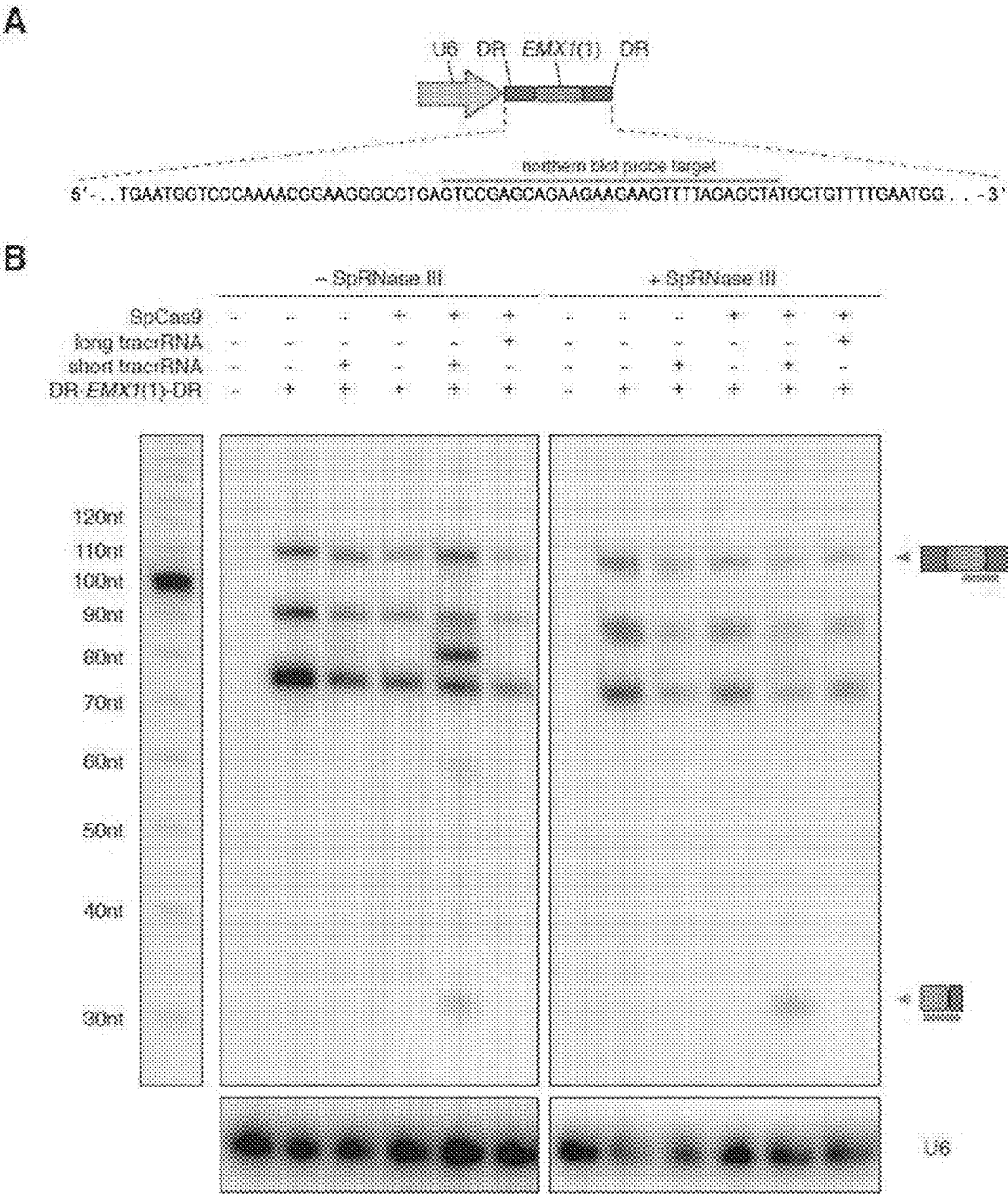


FIG. 9A-B

a

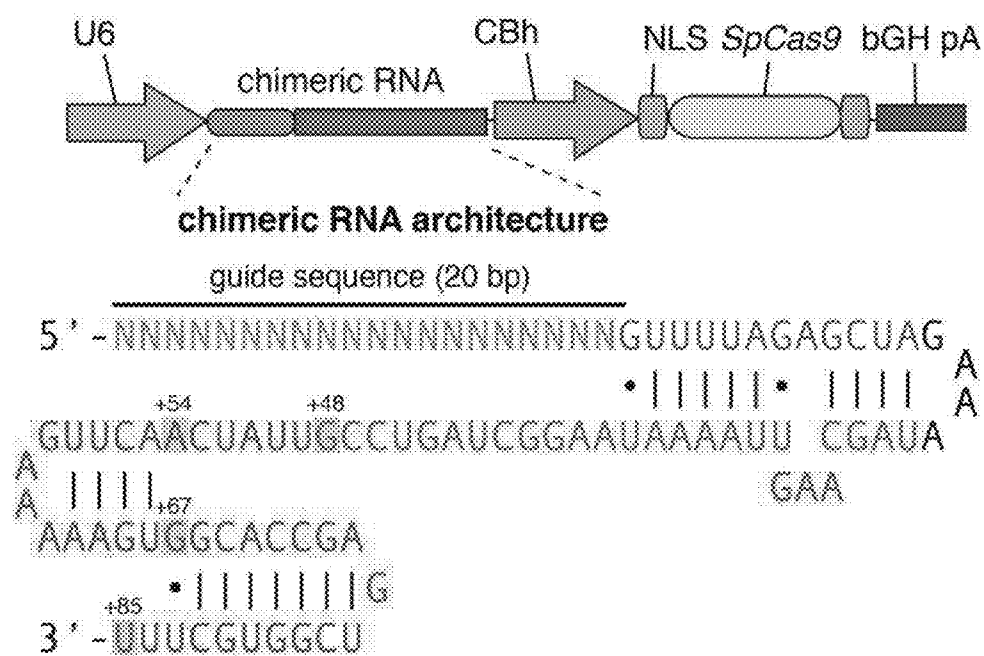


FIG. 10A

b

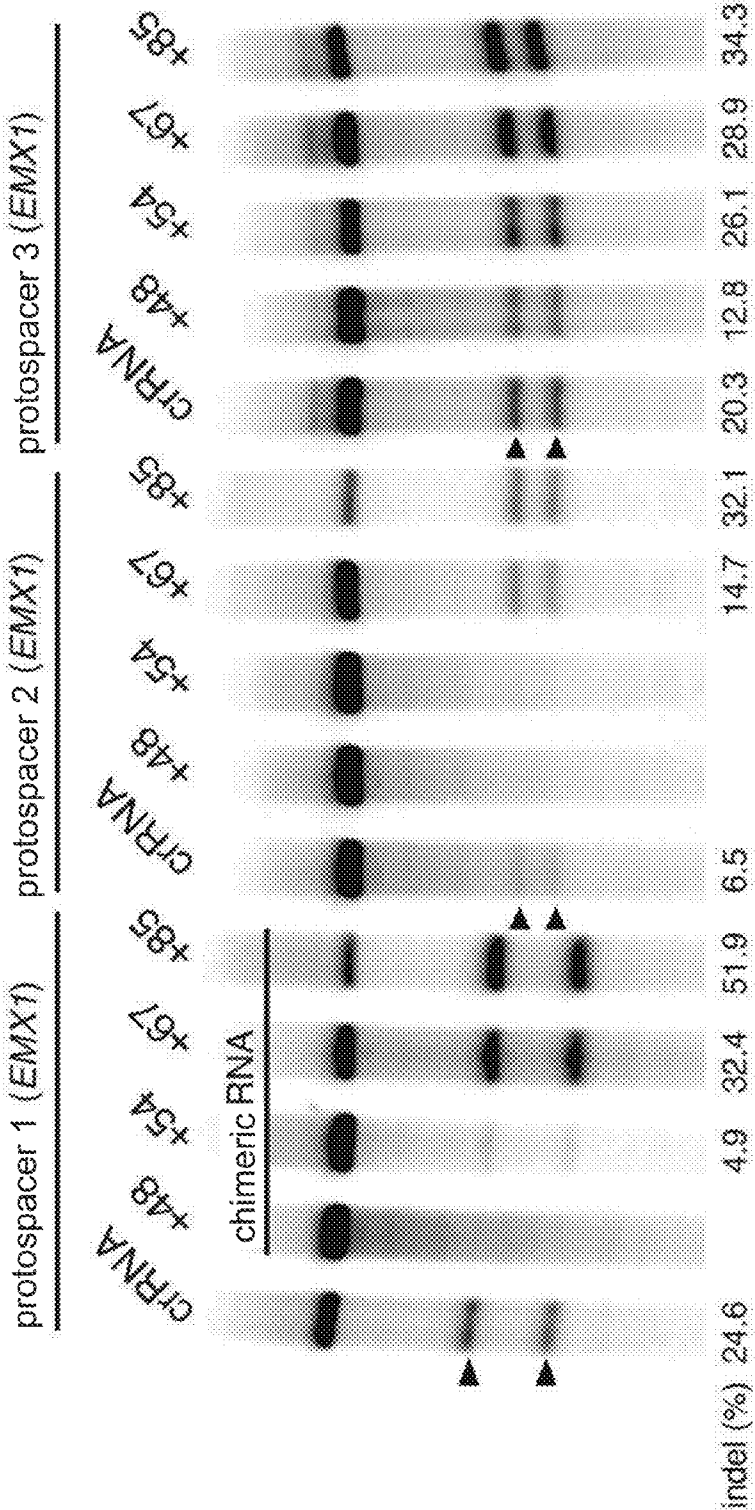


FIG. 10B

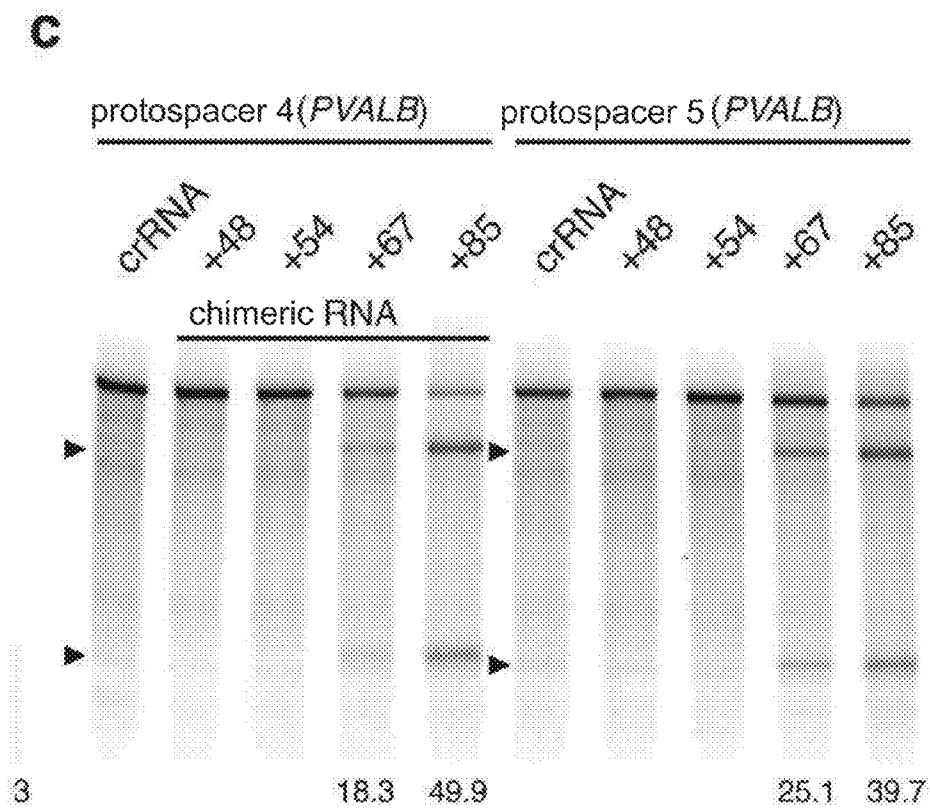


FIG. 10C

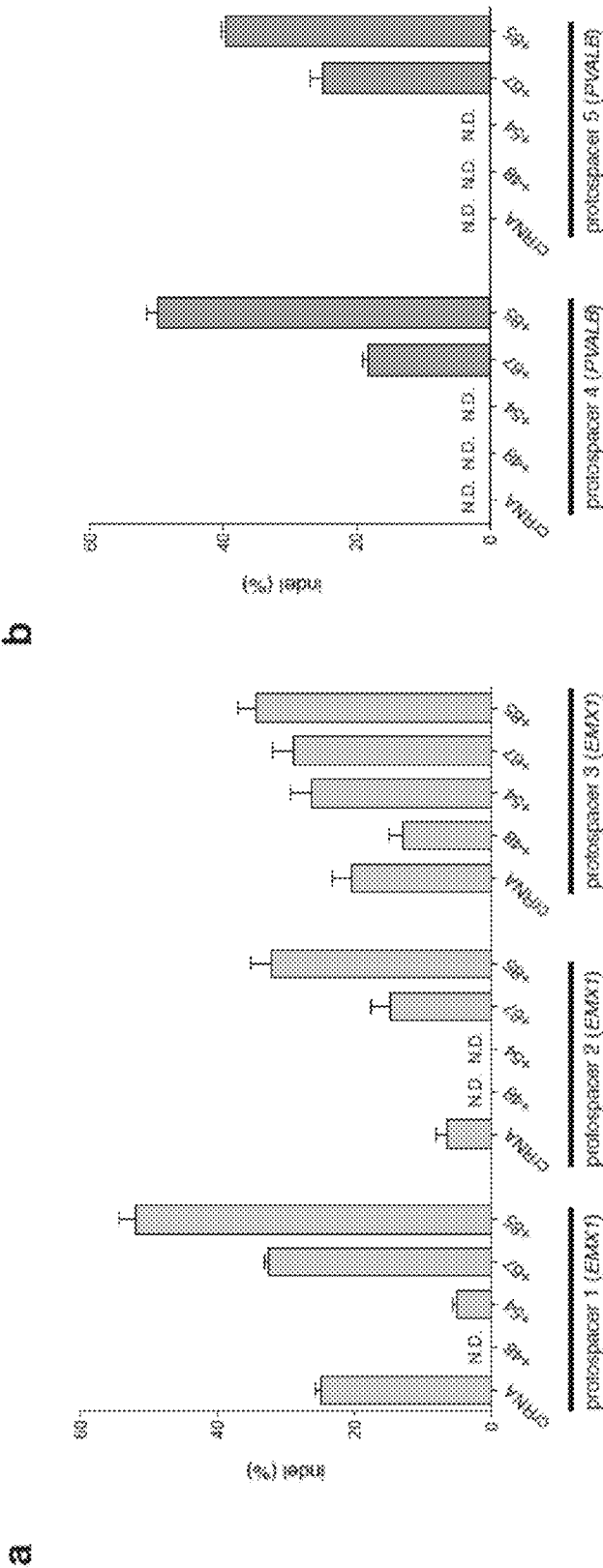


FIG. 11A-B

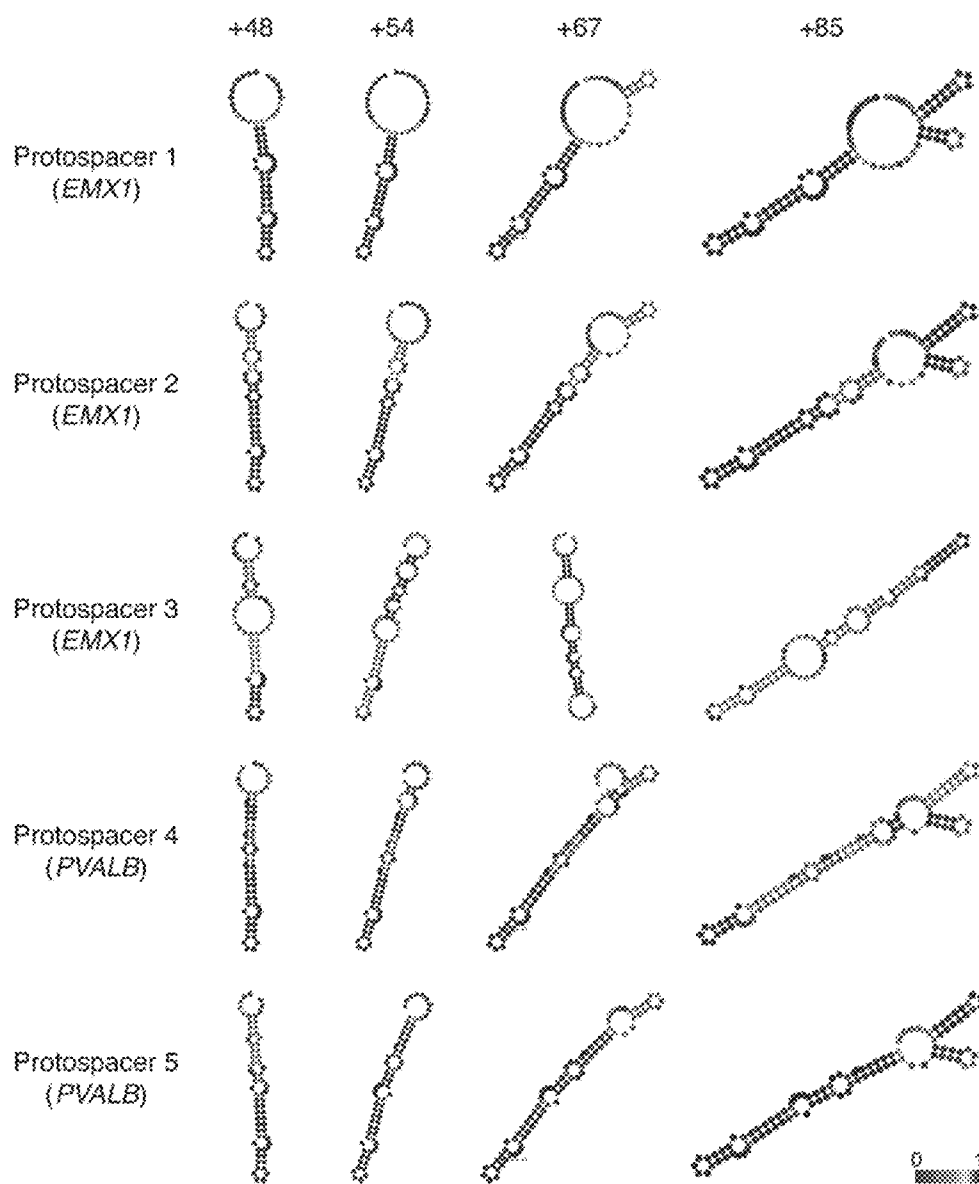


FIG. 12

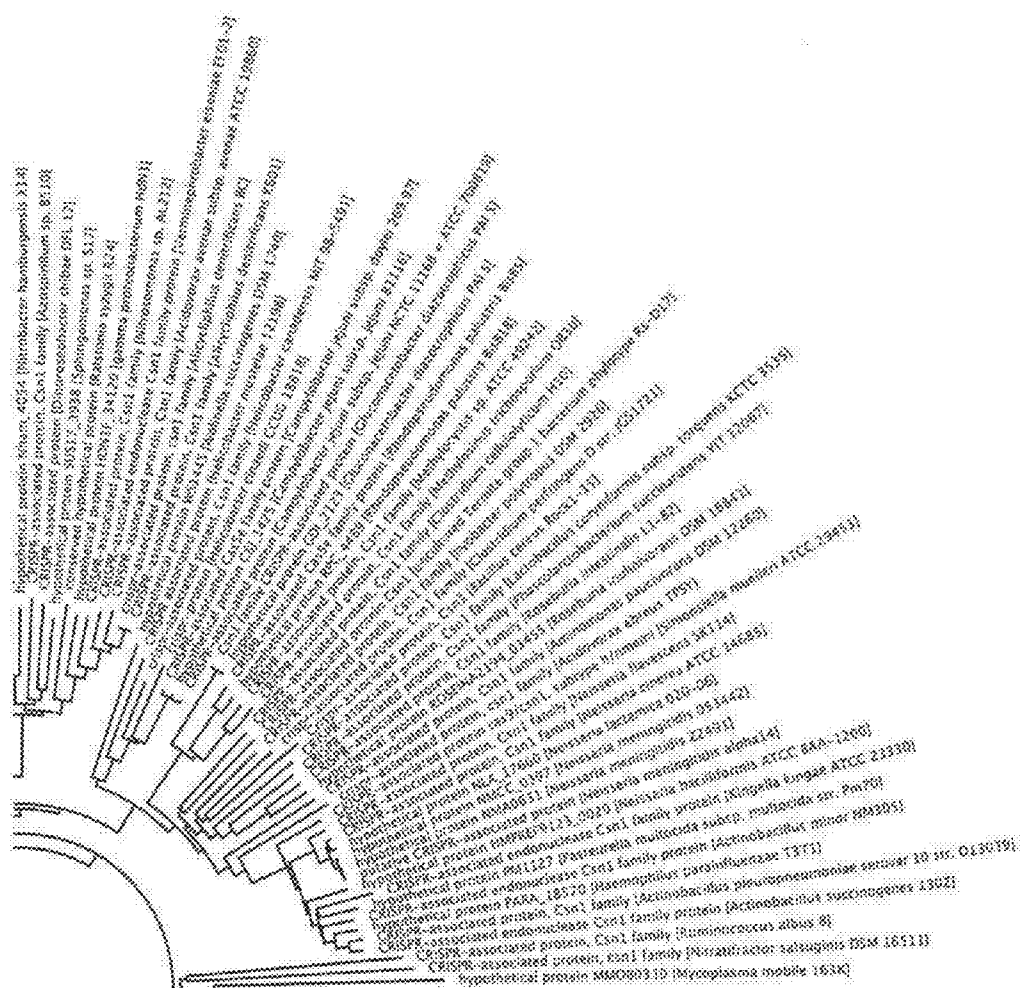


FIG. 13B

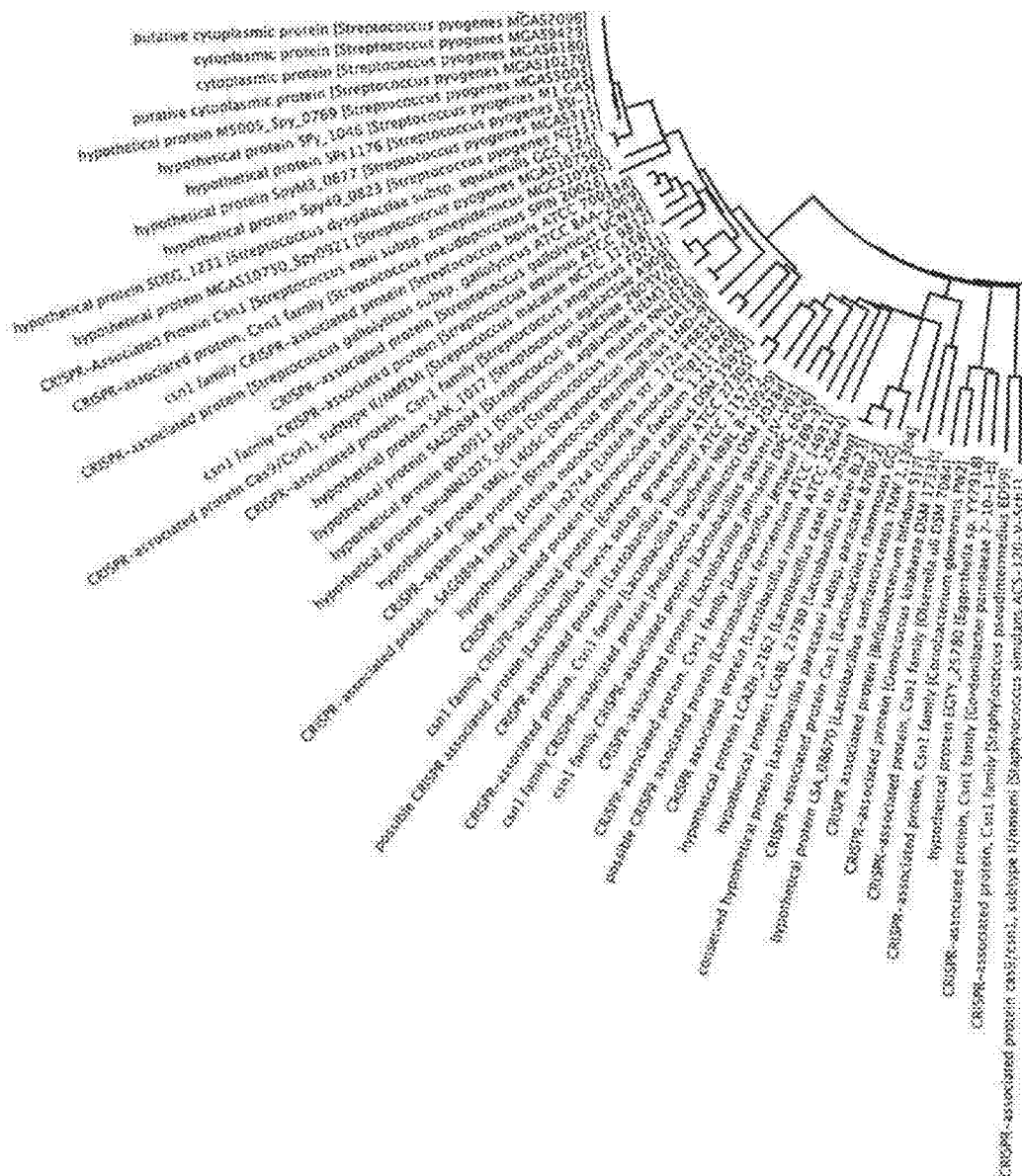


FIG. 13C

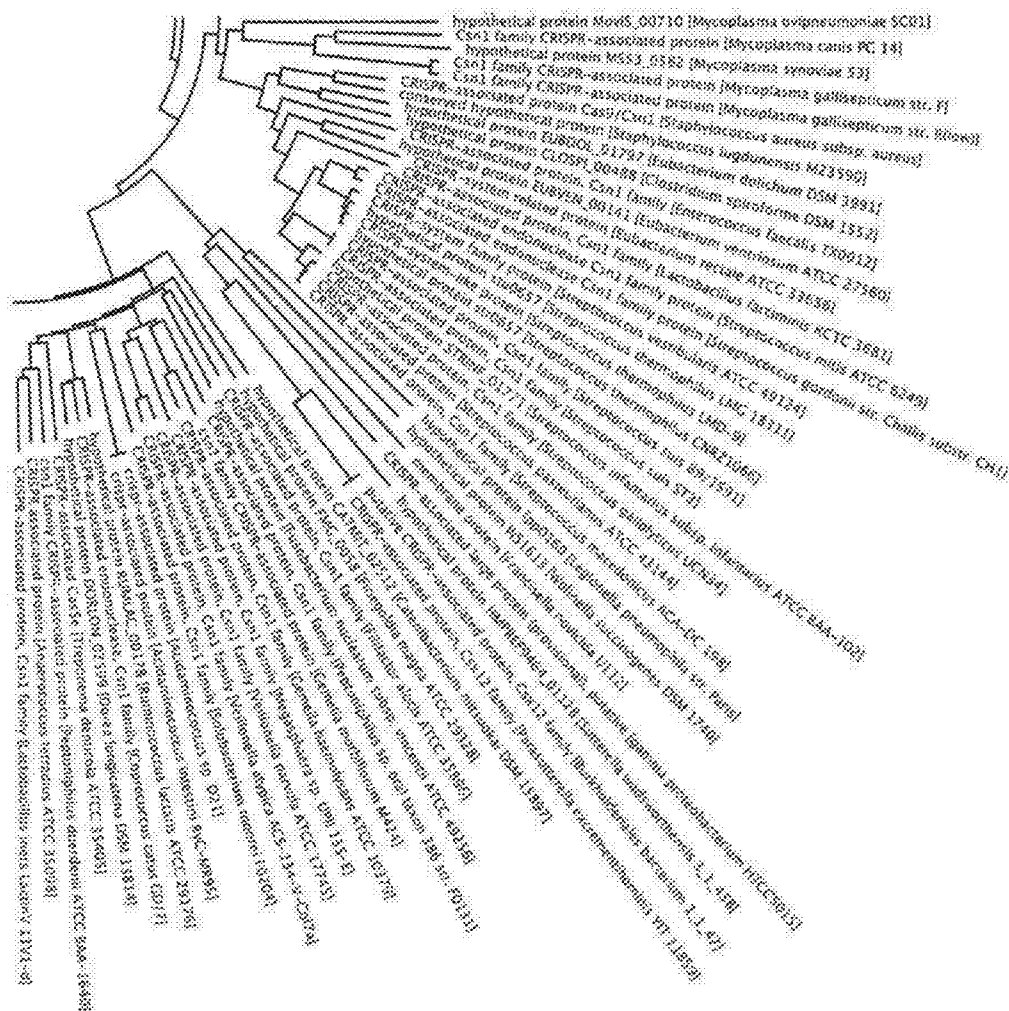
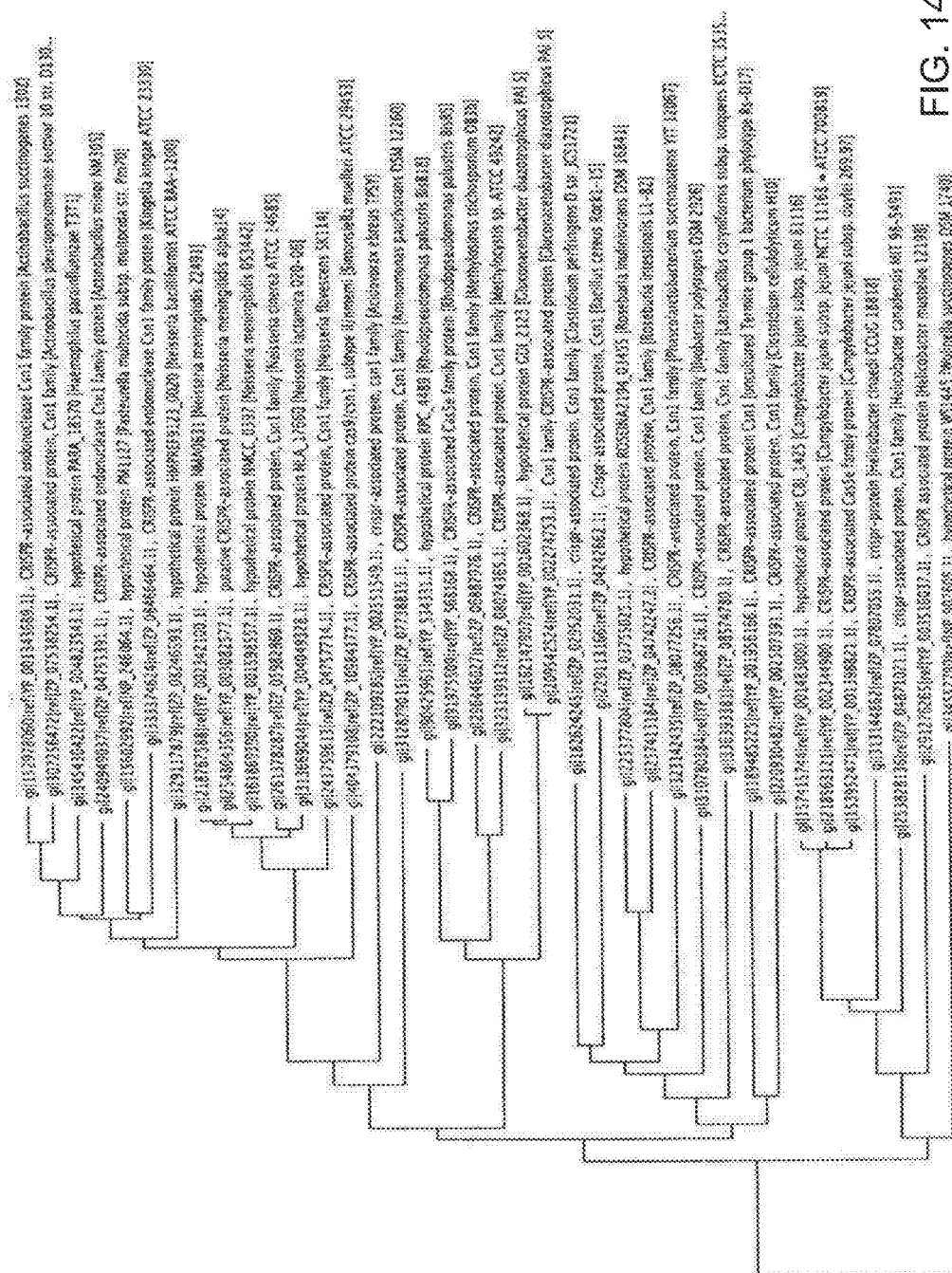
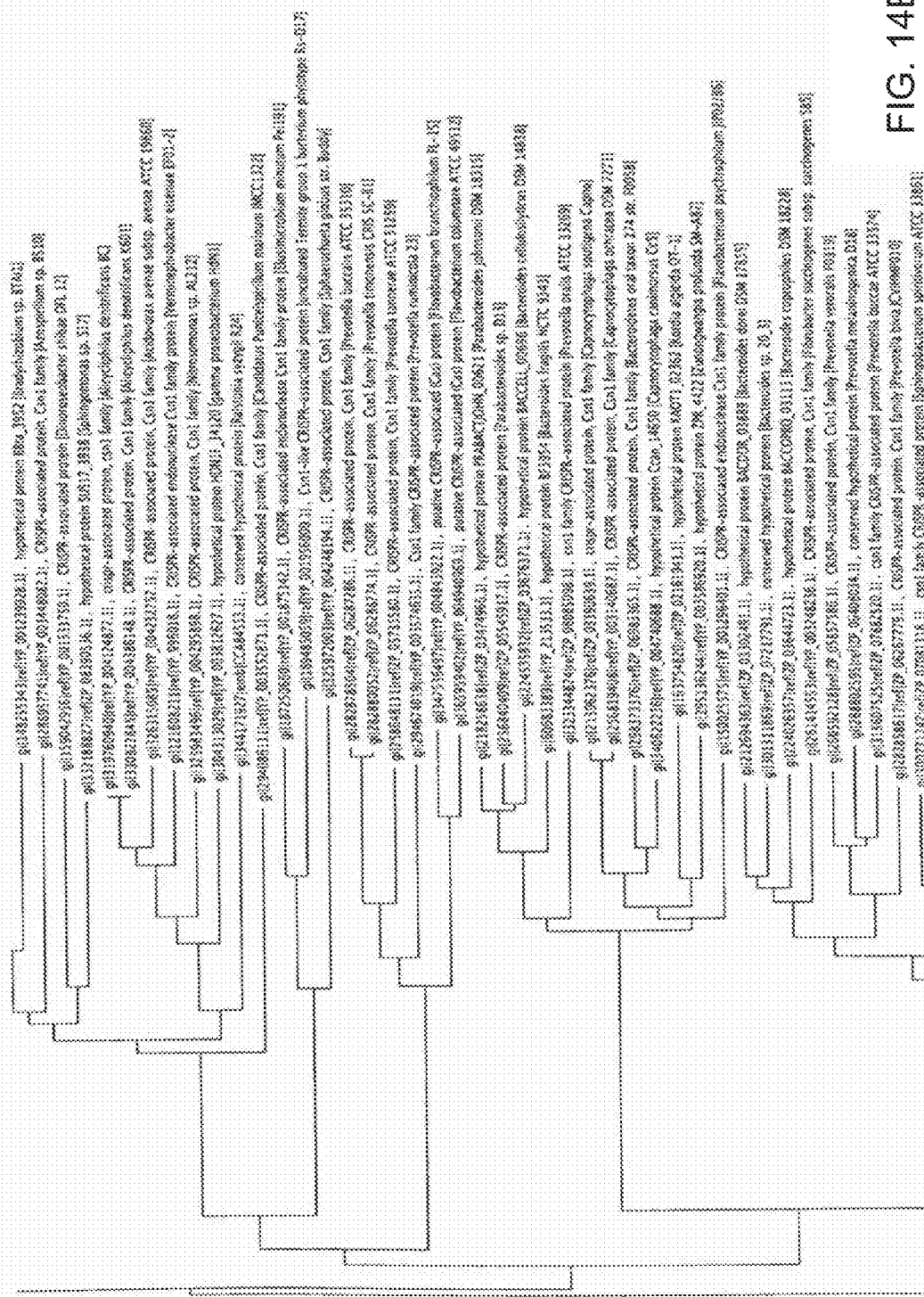
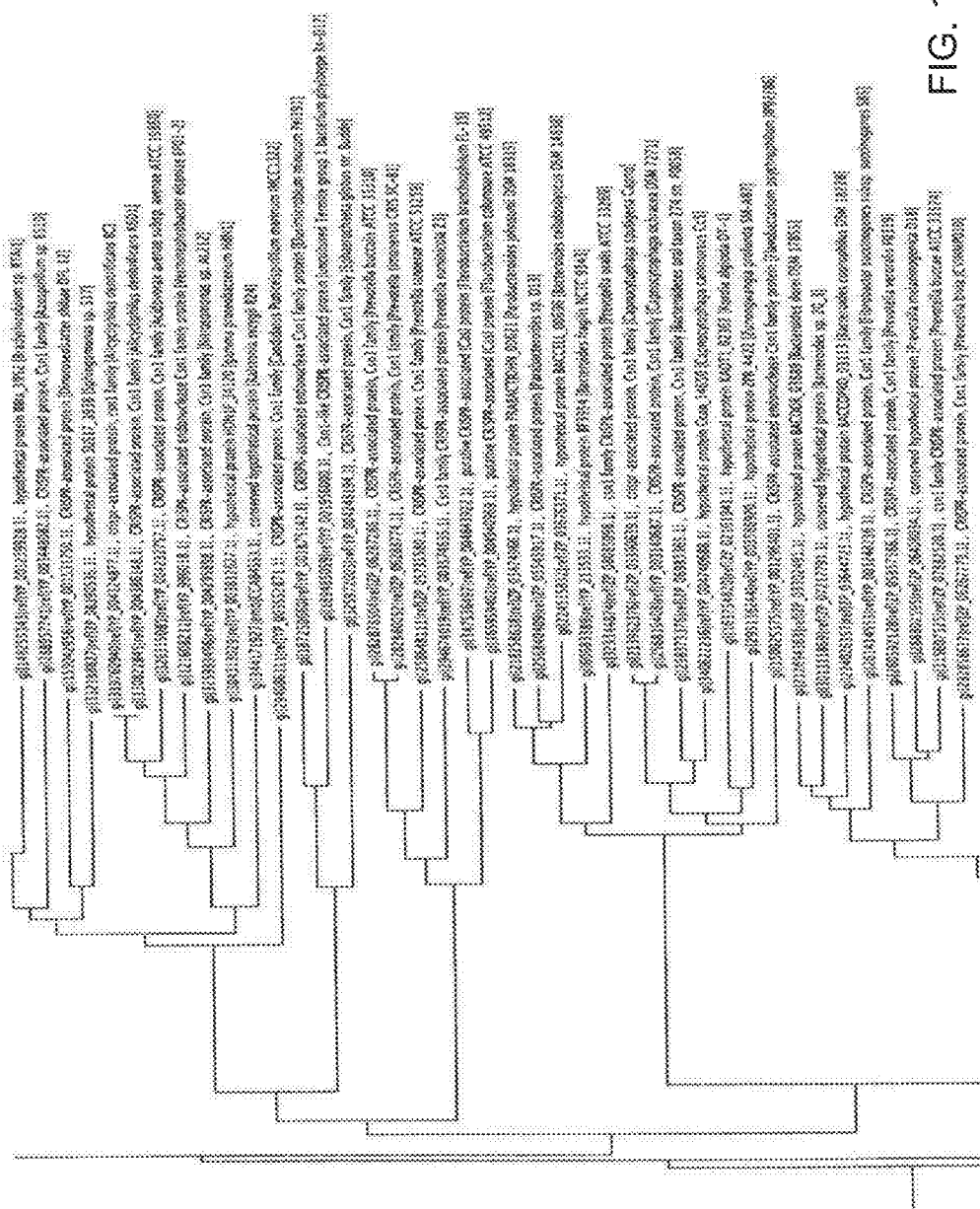
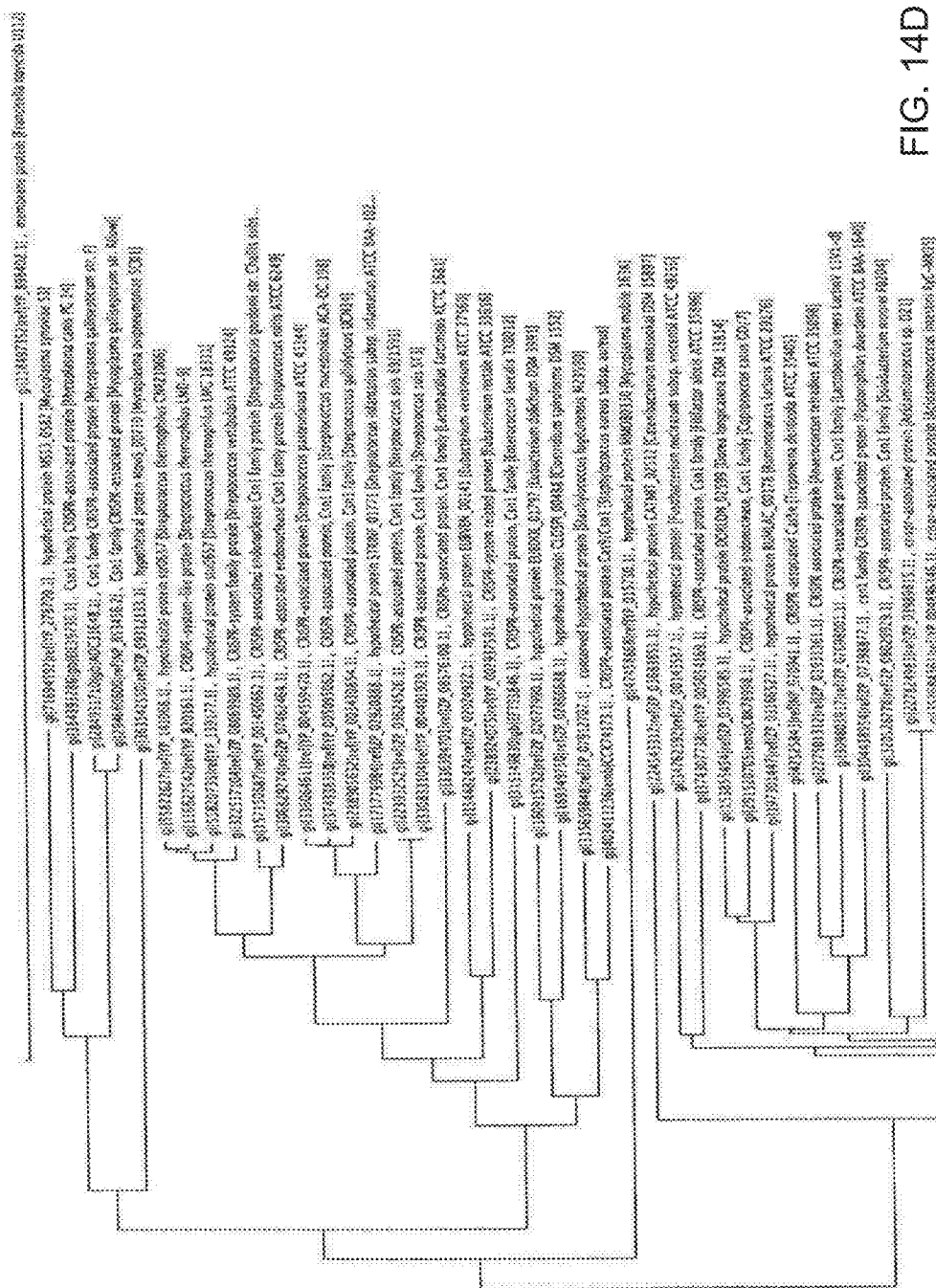


FIG. 13D









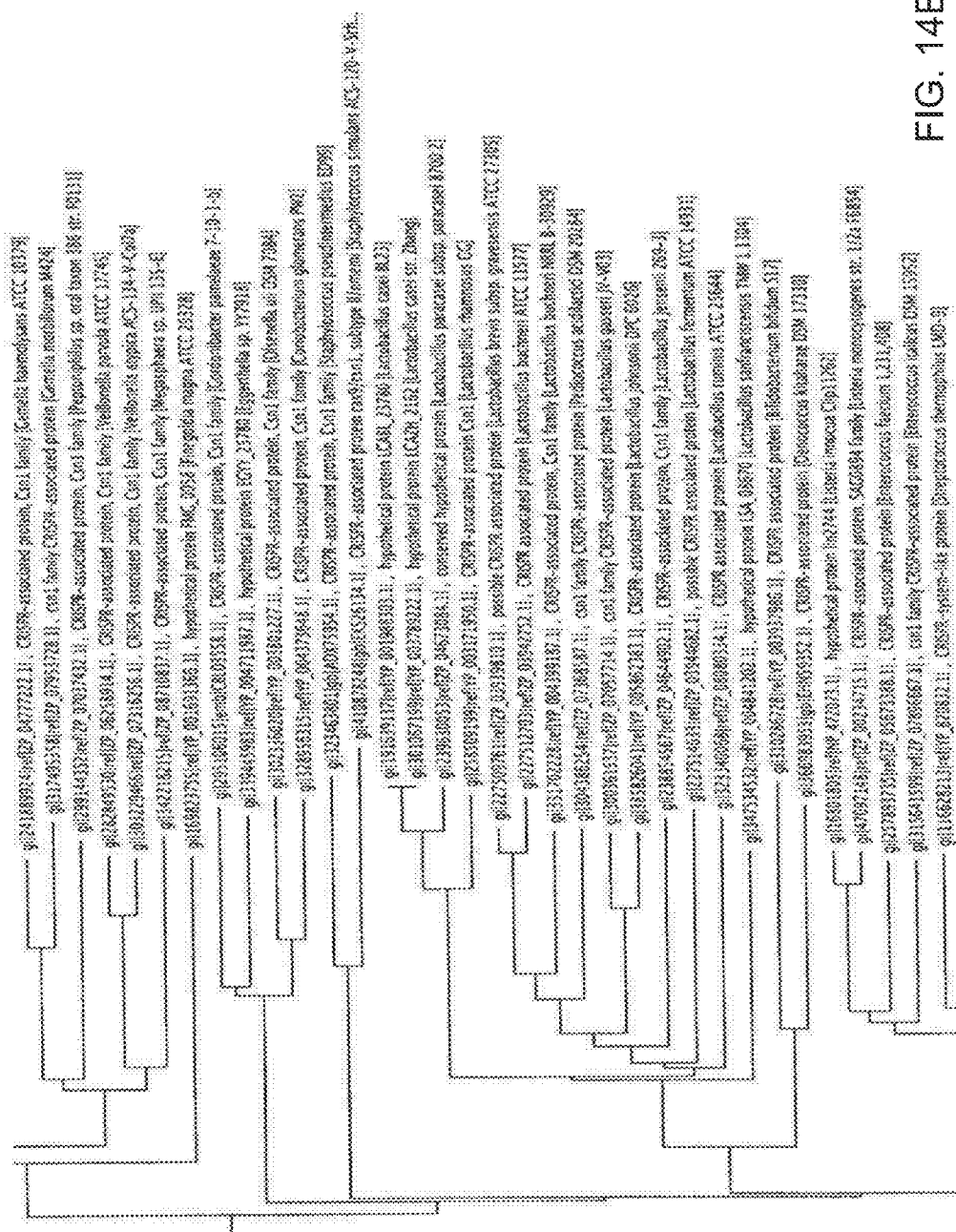


FIG. 14E

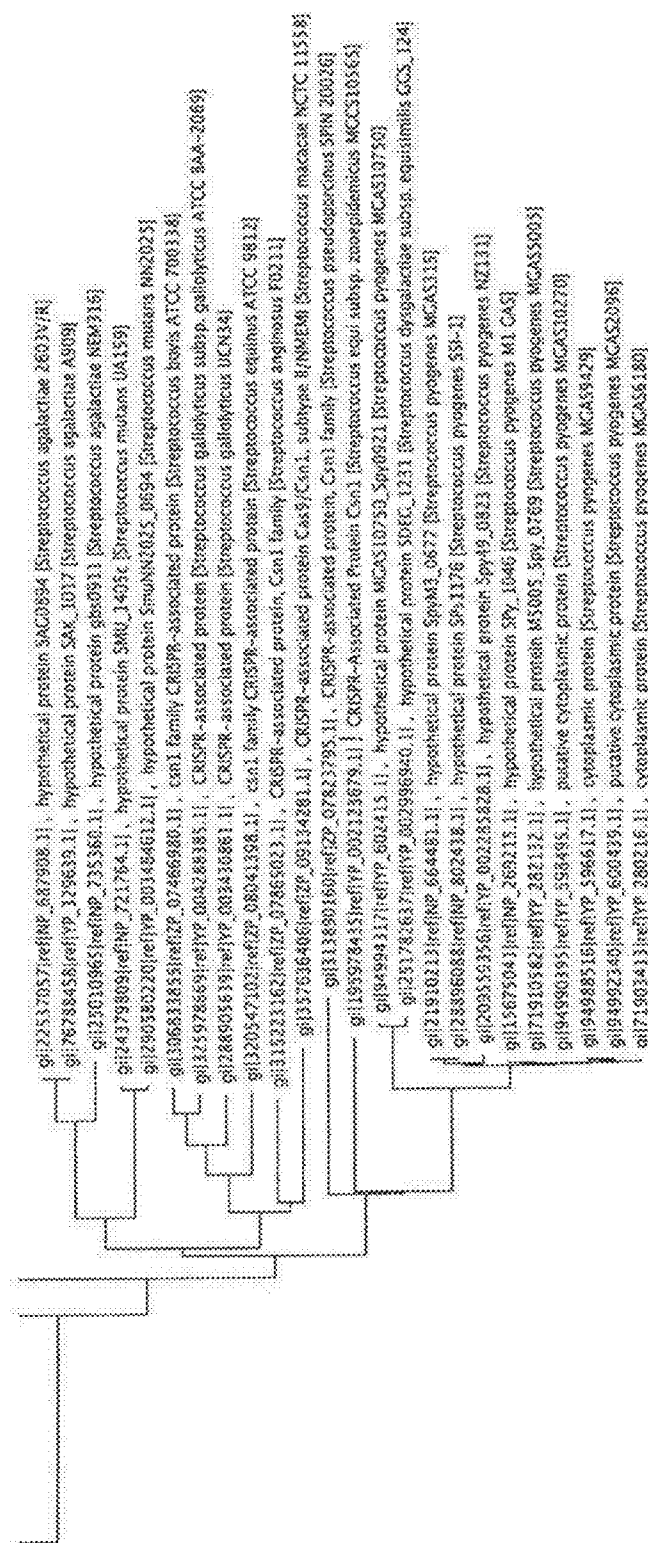


FIG. 14F

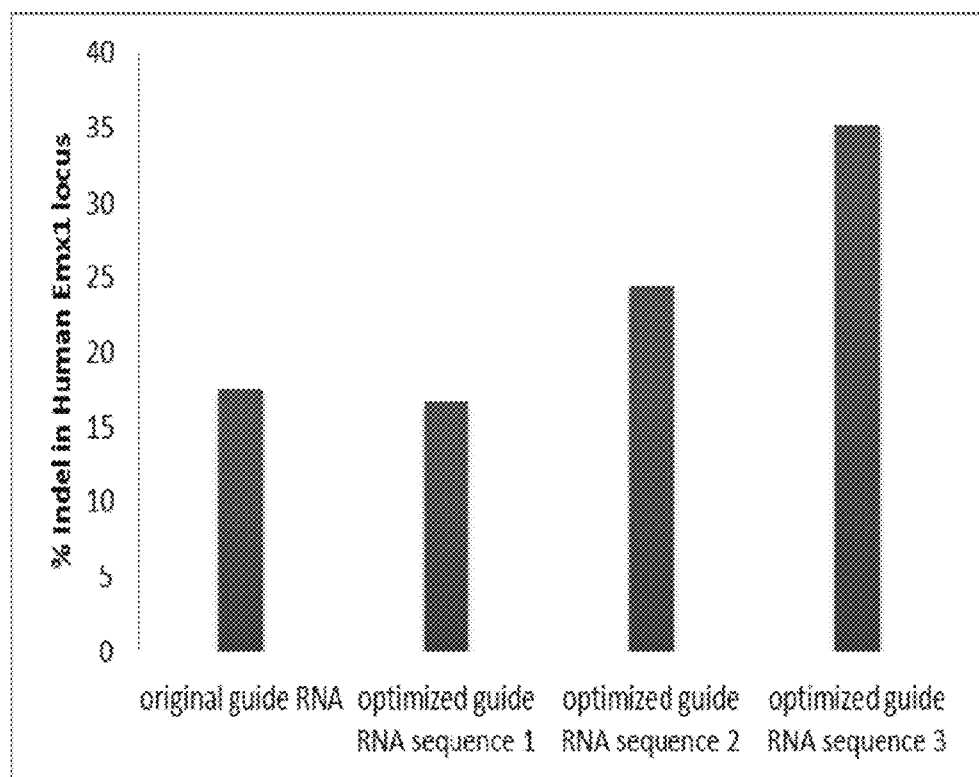
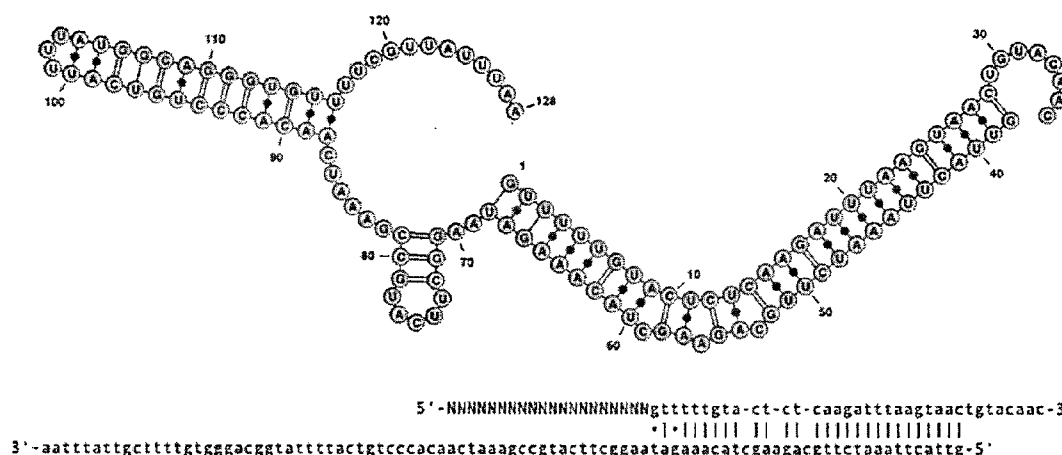


FIG. 15

S. thermophilus CRISPR1 RNA cofold of tracrRNA with direct repeat



chimeric guide RNA design



FIG. 16

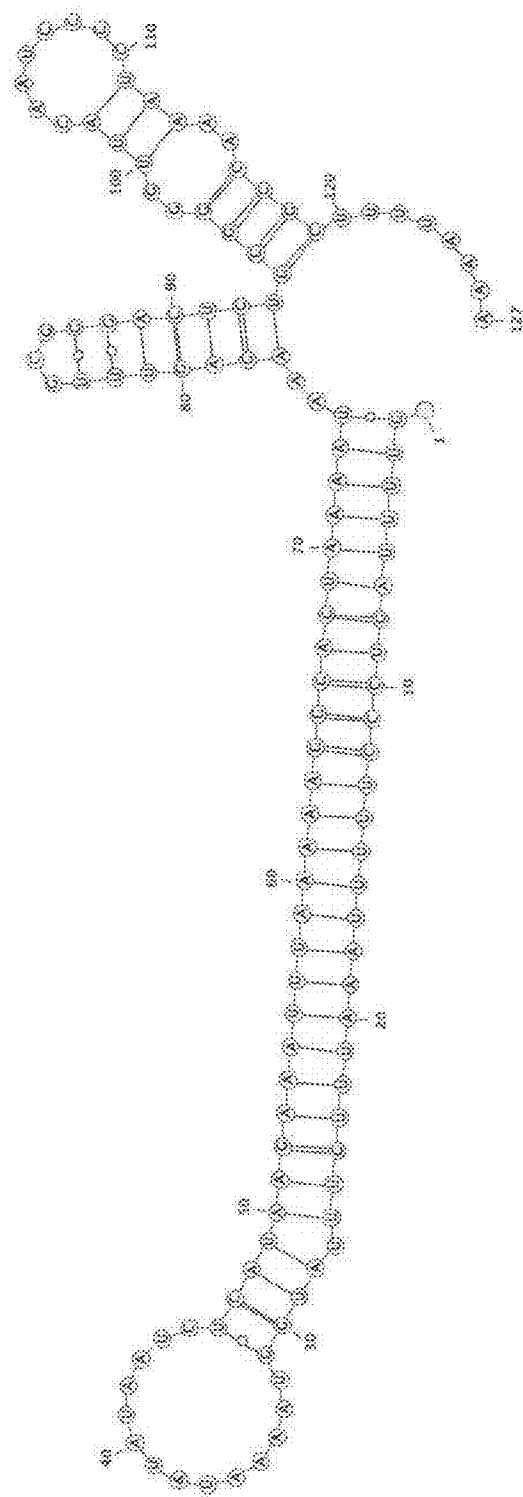
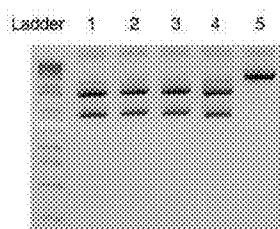


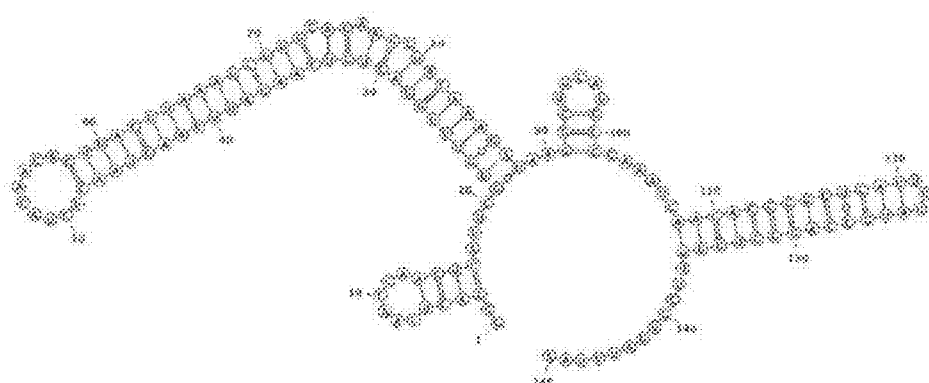
FIG. 17

Stt1Cas9 Test



Lane 1: full tracrRNA + full spacer_DR

full tracrRNA: GUUACUUAUAUUCUUGCAGAAAGCUACAAGAUAAAGGCUUCAUGCCGAAAUCAACACCCUGUCAUUUUUAUGGCAGGGUGUUUUGGUUUUUAA
full spacer_DR: GGGACUCAACCAAGUCAUUCGUUUUUGUACUCUCAAGAUUUUAAGUAACUGUACAAC



Lane 2: mature tracrRNA + mature spacer_DR

mature tracrRNA: CUUSCAGAAAGCUACAAGAUAAAGGCUUCAUGCCGAAAUCAACACCCUGUCAUUUUUAUGGCAGGGUGUUU
mature spacer_DR: GGGACUCAACCAAGUCAUUCGUUUUUGUACUCUCAAGAUUUUAAGUAACUGUACAAC

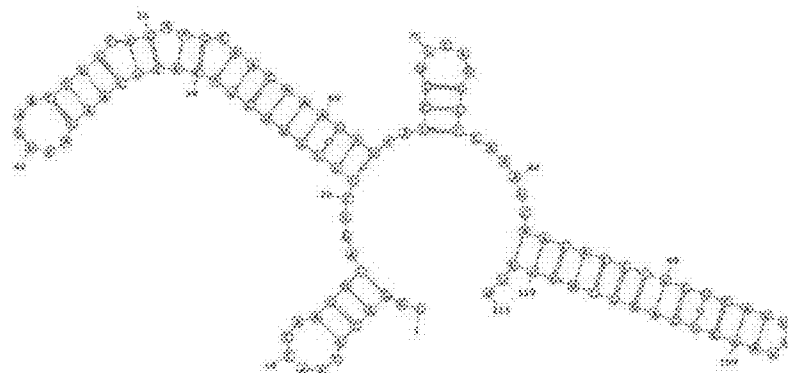
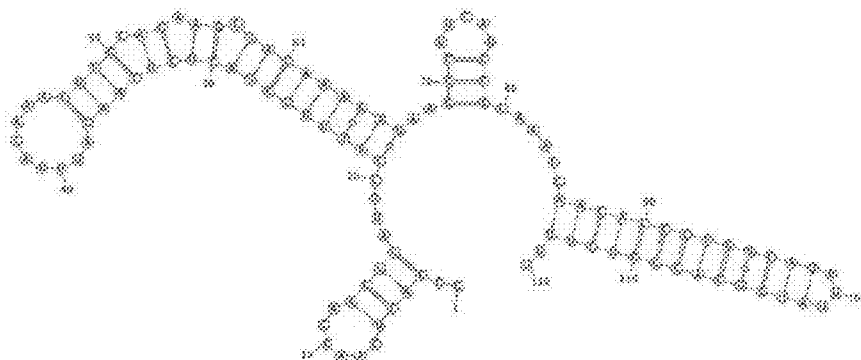


FIG. 18A

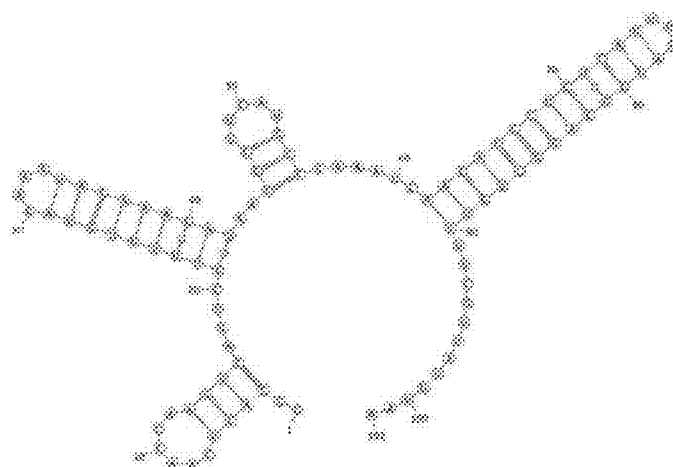
Lane 3: chimeric guideRNA design 1 (1-22 DR, 12-81 tracrRNA)

sequence: GGGACUCAACCAAGUCAUUCGUUUUGUAGUACAAGAUUAGGAACUUCGACAGCUACAAGAUAGGCUUACUGCCGAAAUCAACACCCUGUCAUUUUUAGGCGAGGUGU



Lane 4: chimeric guide RNA design 2 (1-9 DR, 20-91 tracrRNA)

sequence: GGGACUCAACCAAGUCAUUCGUUUUGUAGAAUACAAGAUUAGGCUUACUGCCGAAAUCAACACCCUGUCAUUUUUAGGCGAGGUGUUUUUGUUUUUAA



Lane 5: chimeric guide RNA design 3 (1-9 DR, 20-46 tracrRNA)

sequence: GGGACUCAACCAAGUCAUUCGUUUUGUAGAAUACAAGAUUAGGCUUACUGCCGAA

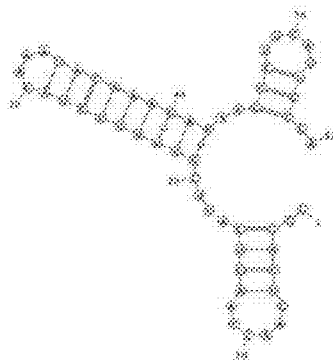
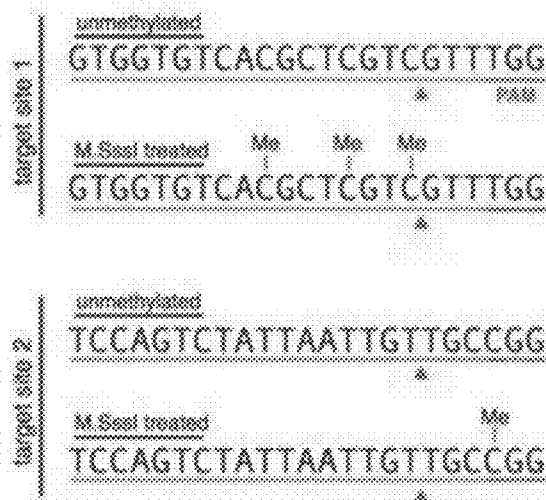


FIG. 18B

a



b

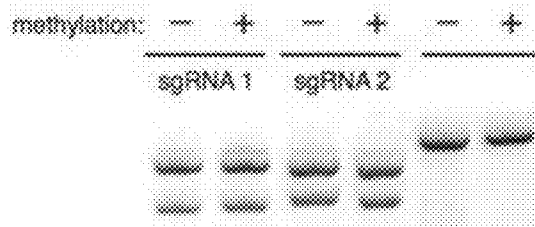
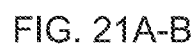


FIG. 19A-B







move	<<<	<<	<	>	>>	>>>	zoom in	1.5x	3x	10x	base	zoom out	1.5x	3x	10x
move	<<<	<<	<	>	>>	>>>	zoom in	1.5x	3x	10x	base	zoom out	1.5x	3x	10x

chr12:2,814,008-2,875,748 | 61,741 bp

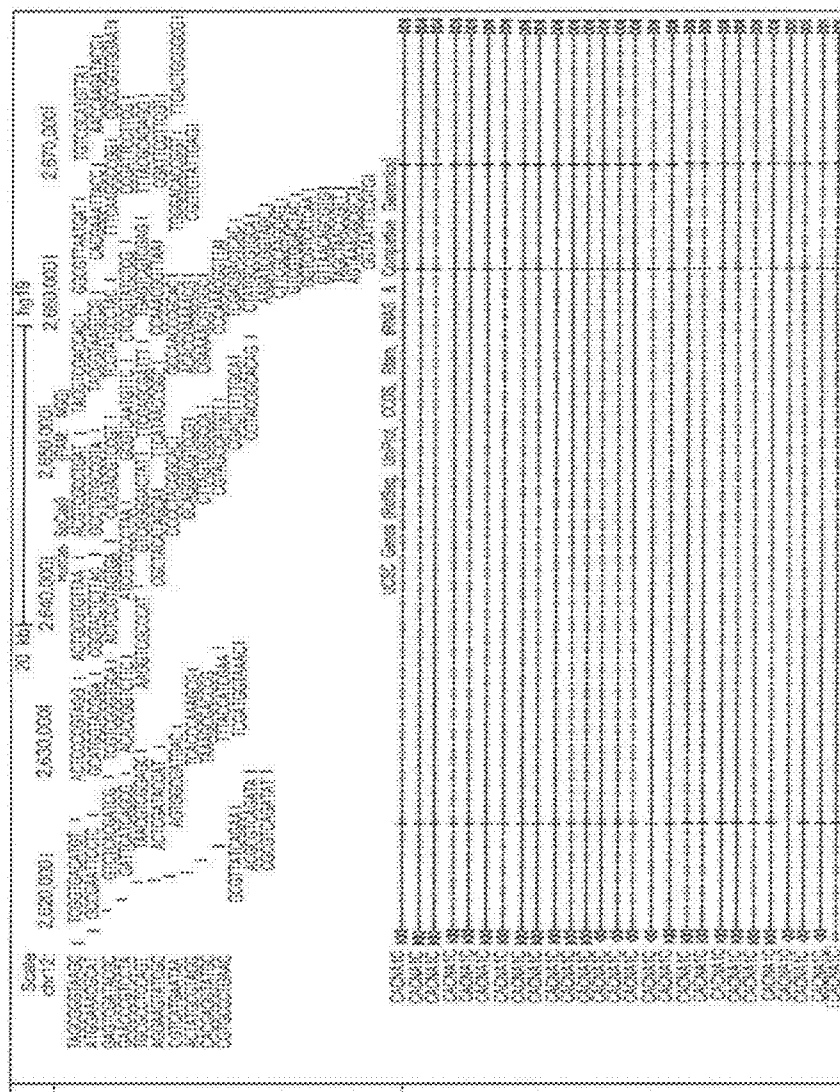
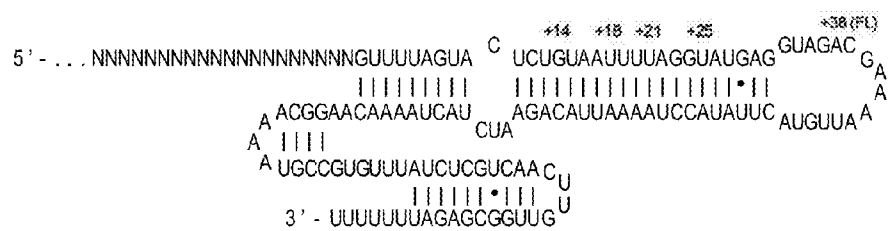
[illegible]

FIG. 22

A



B

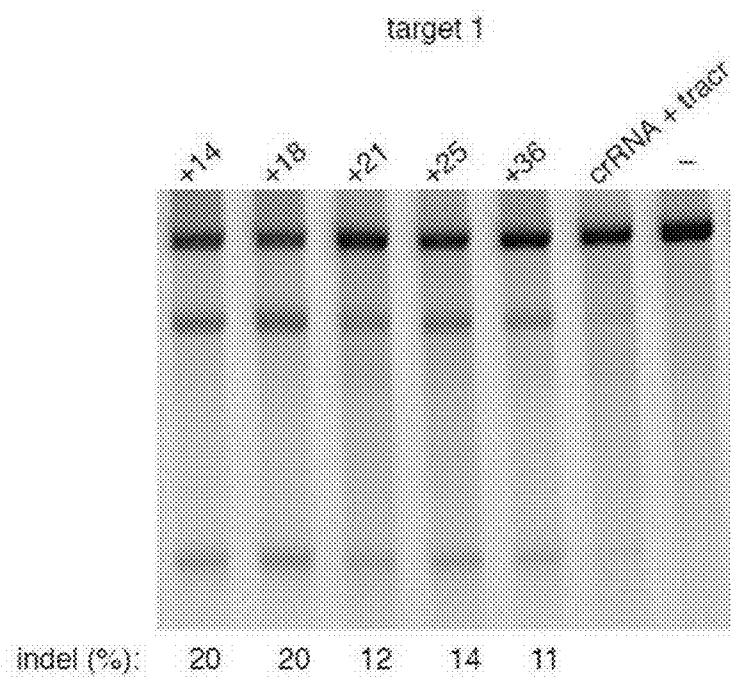


FIG. 23A-B

ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED GUIDE COMPOSITIONS FOR SEQUENCE MANIPULATION

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application is a continuation of International Application No. PCT/US2013/074819 filed Dec. 12, 2013, and published as PCT Publication No. WO 2014/093712 on Jun. 19, 2014 and which claims priority to U.S. provisional patent application 61/836,127 entitled ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED COMPOSITIONS FOR SEQUENCE MANIPULATION filed on Jun. 17, 2013. This application also claims priority to US provisional patent applications 61/758,468; 61/769,046; 61/802,174; 61/806,375; 61/814,263; 61/819,803 and 61/828,130 each entitled ENGINEERING AND OPTIMIZATION OF SYSTEMS, METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION, filed on Jan. 30, 2013; Feb. 25, 2013; Mar. 15, 2013; Mar. 28, 2013; Apr. 20, 2013; May 6, 2013 and May 28, 2013 respectively. Priority is also claimed to US provisional patent applications 61/736,527 and 61/748,427, both entitled SYSTEMS METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION filed on Dec. 12, 2012 and Jan. 2, 2013, respectively. Priority is also claimed to US provisional patent applications 61/791,409 and 61/835,931 both entitled BI-2011/008/44790.02.2003 and BI-2011/008/44790.03.2003 filed on Mar. 15, 2013 and Jun. 17, 2013 respectively.

[0002] Reference is also made to US provisional patent applications 61/835,936, 61/836,101, 61/836,080, 61/836,123 and 61/835,973 each filed Jun. 17, 2013.

[0003] The foregoing applications, and all documents cited therein or during their prosecution (“appln cited documents”) and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein (“herein cited documents”), and all documents cited or referenced in herein cited documents, together with any manufacturer’s instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0004] This invention was made with government support awarded by the National Institutes of Health, NIH Pioneer Award DP1MH100706. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0005] The present invention generally relates to systems, methods and compositions used for the control of gene expression involving sequence targeting, such as genome perturbation or gene-editing, that may use vector systems related to Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and components thereof.

BACKGROUND OF THE INVENTION

[0006] Recent advances in genome sequencing techniques and analysis methods have significantly accelerated the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases. Precise genome targeting technologies are needed to enable systematic reverse engineering of causal genetic variations by allowing selective perturbation of individual genetic elements, as well as to advance synthetic biology, biotechnological, and medical applications. Although genome-editing techniques such as designer zinc fingers, transcription activator-like effectors (TALEs), or homing meganucleases are available for producing targeted genome perturbations, there remains a need for new genome engineering technologies that are affordable, easy to set up, scalable, and amenable to targeting multiple positions within the eukaryotic genome.

SUMMARY OF THE INVENTION

[0007] There exists a pressing need for alternative and robust systems and techniques for sequence targeting with a wide array of applications. This invention addresses this need and provides related advantages. The CRISPR/Cas or the CRISPR-Cas system (both terms are used interchangeably throughout this application) does not require the generation of customized proteins to target specific sequences but rather a single Cas enzyme can be programmed by a short RNA molecule to recognize a specific DNA target, in other words the Cas enzyme can be recruited to a specific DNA target using said short RNA molecule. Adding the CRISPR-Cas system to the repertoire of genome sequencing techniques and analysis methods may significantly simplify the methodology and accelerate the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases. To utilize the CRISPR-Cas system effectively for genome editing without deleterious effects, it is critical to understand aspects of engineering and optimization of these genome engineering tools, which are aspects of the claimed invention.

[0008] In one aspect, the invention provides a vector system comprising one or more vectors. In some embodiments, the system comprises: (a) a first regulatory element operably linked to a tracr mate sequence and one or more insertion sites for inserting one or more guide sequences upstream of the tracr mate sequence, wherein when expressed, the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a cell, e.g., eukaryotic cell, wherein the CRISPR complex comprises a CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence; and (b) a second regulatory element operably linked to an enzyme-coding sequence encoding said CRISPR enzyme comprising a nuclear localization sequence; wherein components (a) and (b) are located on the same or different vectors of the system. In some embodiments, component (a) further comprises the tracr sequence downstream of the tracr mate sequence under the control of the first regulatory element. In some embodiments, component (a) further comprises two or more guide sequences operably linked to the first regulatory element, wherein when expressed, each of the two or more guide sequences direct sequence specific binding of a CRISPR complex to a different target sequence in a eukaryotic cell. In some embodiments, the system comprises the tracr sequence

under the control of a third regulatory element, such as a polymerase III promoter. In some embodiments, the tracer sequence exhibits at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of sequence complementarity along the length of the tracer mate sequence when optimally aligned. In some embodiments, the CRISPR complex comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of said CRISPR complex in a detectable amount in the nucleus of a eukaryotic cell. Without wishing to be bound by theory, it is believed that a nuclear localization sequence is not necessary for CRISPR complex activity in eukaryotes, but that including such sequences enhances activity of the system, especially as to targeting nucleic acid molecules in the nucleus. In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is *S. pneumoniae*, *S. pyogenes*, or *S. thermophilus* Cas9, and may include mutated Cas9 derived from these organisms. The enzyme may be a Cas9 homolog or ortholog. In some embodiments, the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell. In some embodiments, the CRISPR enzyme directs cleavage of one or two strands at the location of the target sequence. In some embodiments, the CRISPR enzyme lacks DNA strand cleavage activity. In some embodiments, the first regulatory element is a polymerase III promoter. In some embodiments, the second regulatory element is a polymerase II promoter. In some embodiments, the guide sequence is at least 15, 16, 17, 18, 19, 20, 25 nucleotides, or between 10-30, or between 15-25, or between 15-20 nucleotides in length. In general, and throughout this specification, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (e.g. circular); nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g. retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses). Viral vectors also include polynucleotides carried by a virus for transfection into a host cell. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g. bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively-linked. Such vectors are referred to herein as “expression vectors.” Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[0009] Recombinant expression vectors can comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory

elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence (e.g. in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell).

[0010] The term “regulatory element” is intended to include promoters, enhancers, internal ribosomal entry sites (IRES), and other expression control elements (e.g. transcription termination signals, such as polyadenylation signals and poly-U sequences). Such regulatory elements are described, for example, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory elements include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). A tissue-specific promoter may direct expression primarily in a desired tissue of interest, such as muscle, neuron, bone, skin, blood, specific organs (e.g. liver, pancreas), or particular cell types (e.g. lymphocytes). Regulatory elements may also direct expression in a temporal-dependent manner, such as in a cell-cycle dependent or developmental stage-dependent manner, which may or may not also be tissue or cell-type specific. In some embodiments, a vector comprises one or more pol III promoter (e.g. 1, 2, 3, 4, 5, or more pol III promoters), one or more pol II promoters (e.g. 1, 2, 3, 4, 5, or more pol II promoters), one or more pol I promoters (e.g. 1, 2, 3, 4, 5, or more pol I promoters), or combinations thereof. Examples of pol III promoters include, but are not limited to, U6 and H1 promoters. Examples of pol II promoters include, but are not limited to, the retroviral Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), the cytomegalovirus (CMV) promoter (optionally with the CMV enhancer) [see, e.g., Boshart et al, Cell, 41:521-530 (1985)], the SV40 promoter, the dihydrofolate reductase promoter, the p-actin promoter, the phosphoglycerol kinase (PGK) promoter, and the EF a promoter. Also encompassed by the term “regulatory element” are enhancer elements, such as WPRE; CMV enhancers; the R-U5' segment in LTR of HTLV-I (Mol. Cell. Biol., Vol. 8(1), p. 466-472, 1988); SV40 enhancer; and the intron sequence between exons 2 and 3 of rabbit 3-globin (Proc. Natl. Acad. Sci. USA., Vol. 78(3), p. 1527-31, 1981). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression desired, etc. A vector can be introduced into host cells to thereby produce transcripts, proteins, or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (e.g., clustered regularly interspersed short palindromic repeats (CRISPR) transcripts, proteins, enzymes, mutant forms thereof, fusion proteins thereof, etc.).

[0011] Advantageous vectors include lentiviruses and adeno-associated viruses, and types of such vectors can also be selected for targeting particular types of cells.

[0012] In one aspect, the invention provides a vector comprising a regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising one or more nuclear localization sequences. In some embodi-

ments, said regulatory element drives transcription of the CRISPR enzyme in a eukaryotic cell such that said CRISPR enzyme accumulates in a detectable amount in the nucleus of the eukaryotic cell. In some embodiments, the regulatory element is a polymerase II promoter. In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is *S. pneumoniae*, *S. pyogenes* or *S. thermophilus* Cas9, and may include mutated Cas9 derived from these organisms. In some embodiments, the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell. In some embodiments, the CRISPR enzyme directs cleavage of one or two strands at the location of the target sequence. In some embodiments, the CRISPR enzyme lacks DNA strand cleavage activity.

[0013] In one aspect, the invention provides a CRISPR enzyme comprising one or more nuclear localization sequences of sufficient strength to drive accumulation of said CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell. In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is *S. pneumoniae*, *S. pyogenes* or *S. thermophilus* Cas9, and may include mutated Cas9 derived from these organisms. The enzyme may be a Cas9 homolog or ortholog. In some embodiments, the CRISPR enzyme lacks the ability to cleave one or more strands of a target sequence to which it binds.

[0014] In one aspect, the invention provides a eukaryotic host cell comprising (a) a first regulatory element operably linked to a tracr mate sequence and one or more insertion sites for inserting one or more guide sequences upstream of the tracr mate sequence, wherein when expressed, the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a eukaryotic cell, wherein the CRISPR complex comprises a CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence, and/or (b) a second regulatory element operably linked to an enzyme-coding sequence encoding said CRISPR enzyme comprising a nuclear localization sequence. In some embodiments, the host cell comprises components (a) and (b). In some embodiments, component (a), component (b), or components (a) and (b) are stably integrated into a genome of the host eukaryotic cell. In some embodiments, component (a) further comprises the tracr sequence downstream of the tracr mate sequence under the control of the first regulatory element. In some embodiments, component (a) further comprises two or more guide sequences operably linked to the first regulatory element, wherein when expressed, each of the two or more guide sequences direct sequence specific binding of a CRISPR complex to a different target sequence in a eukaryotic cell. In some embodiments, the eukaryotic host cell further comprises a third regulatory element, such as a polymerase III promoter, operably linked to said tracr sequence. In some embodiments, the tracr sequence exhibits at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned. In some embodiments, the CRISPR enzyme comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of said CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell. In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In

some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is *S. pneumoniae*, *S. pyogenes* or *S. thermophilus* Cas9, and may include mutated Cas9 derived from these organisms. The enzyme may be a Cas9 homolog or ortholog. In some embodiments, the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell. In some embodiments, the CRISPR enzyme directs cleavage of one or two strands at the location of the target sequence. In some embodiments, the CRISPR enzyme lacks DNA strand cleavage activity. In some embodiments, the first regulatory element is a polymerase III promoter. In some embodiments, the second regulatory element is a polymerase II promoter. In some embodiments, the guide sequence is at least 15, 16, 17, 18, 19, 20, 25 nucleotides, or between 10-30, or between 15-25, or between 15-20 nucleotides in length. In an aspect, the invention provides a non-human eukaryotic organism; preferably a multicellular eukaryotic organism, comprising a eukaryotic host cell according to any of the described embodiments. In other aspects, the invention provides a eukaryotic organism; preferably a multicellular eukaryotic organism, comprising a eukaryotic host cell according to any of the described embodiments. The organism in some embodiments of these aspects may be an animal; for example a mammal. Also, the organism may be an arthropod such as an insect. The organism also may be a plant. Further, the organism may be a fungus.

[0015] In one aspect, the invention provides a kit comprising one or more of the components described herein. In some embodiments, the kit comprises a vector system and instructions for using the kit. In some embodiments, the vector system comprises (a) a first regulatory element operably linked to a tracr mate sequence and one or more insertion sites for inserting one or more guide sequences upstream of the tracr mate sequence, wherein when expressed, the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a eukaryotic cell, wherein the CRISPR complex comprises a CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence; and/or (b) a second regulatory element operably linked to an enzyme-coding sequence encoding said CRISPR enzyme comprising a nuclear localization sequence. In some embodiments, the kit comprises components (a) and (b) located on the same or different vectors of the system. In some embodiments, component (a) further comprises the tracr sequence downstream of the tracr mate sequence under the control of the first regulatory element. In some embodiments, component (a) further comprises two or more guide sequences operably linked to the first regulatory element, wherein when expressed, each of the two or more guide sequences direct sequence specific binding of a CRISPR complex to a different target sequence in a eukaryotic cell. In some embodiments, the system further comprises a third regulatory element, such as a polymerase III promoter, operably linked to said tracr sequence. In some embodiments, the tracr sequence exhibits at least 50%, 60%, 70%, 80%, 90%, 95%, or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned. In some embodiments, the CRISPR enzyme comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of said CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell. In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In

some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is *S. pneumoniae*, *S. pyogenes* or *S. thermophilus* Cas9, and may include mutated Cas9 derived from these organisms. The enzyme may be a Cas9 homolog or ortholog. In some embodiments, the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell. In some embodiments, the CRISPR enzyme directs cleavage of one or two strands at the location of the target sequence. In some embodiments, the CRISPR enzyme lacks DNA strand cleavage activity. In some embodiments, the first regulatory element is a polymerase III promoter. In some embodiments, the second regulatory element is a polymerase II promoter. In some embodiments, the guide sequence is at least 15, 16, 17, 18, 19, 20, 25 nucleotides, or between 10-30, or between 15-25, or between 15-20 nucleotides in length.

[0016] In one aspect, the invention provides a method of modifying a target polynucleotide in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the target polynucleotide to effect cleavage of said target polynucleotide thereby modifying the target polynucleotide, wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said target polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence. In some embodiments, said cleavage comprises cleaving one or two strands at the location of the target sequence by said CRISPR enzyme. In some embodiments, said cleavage results in decreased transcription of a target gene. In some embodiments, the method further comprises repairing said cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide. In some embodiments, said mutation results in one or more amino acid changes in a protein expressed from a gene comprising the target sequence. In some embodiments, the method further comprises delivering one or more vectors to said eukaryotic cell, wherein the one or more vectors drive expression of one or more of: the CRISPR enzyme, the guide sequence linked to the tracr mate sequence, and the tracr sequence. In some embodiments, said vectors are delivered to the eukaryotic cell in a subject. In some embodiments, said modifying takes place in said eukaryotic cell in a cell culture. In some embodiments, the method further comprises isolating said eukaryotic cell from a subject prior to said modifying. In some embodiments, the method further comprises returning said eukaryotic cell and/or cells derived therefrom to said subject.

[0017] In one aspect, the invention provides a method of modifying expression of a polynucleotide in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the polynucleotide such that said binding results in increased or decreased expression of said polynucleotide; wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence. In some embodiments, the method further comprises delivering one or more vectors to said eukaryotic cells, wherein the one or more vectors drive

expression of one or more of: the CRISPR enzyme, the guide sequence linked to the tracr mate sequence, and the tracr sequence.

[0018] In one aspect, the invention provides a method of generating a model eukaryotic cell comprising a mutated disease gene. In some embodiments, a disease gene is any gene associated with an increase in the risk of having or developing a disease. In some embodiments, the method comprises (a) introducing one or more vectors into a eukaryotic cell, wherein the one or more vectors drive expression of one or more of: a CRISPR enzyme, a guide sequence linked to a tracr mate sequence, and a tracr sequence; and (b) allowing a CRISPR complex to bind to a target polynucleotide to effect cleavage of the target polynucleotide within said disease gene, wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence within the target polynucleotide, and (2) the tracr mate sequence that is hybridized to the tracr sequence, thereby generating a model eukaryotic cell comprising a mutated disease gene. In some embodiments, said cleavage comprises cleaving one or two strands at the location of the target sequence by said CRISPR enzyme. In some embodiments, said cleavage results in decreased transcription of a target gene. In some embodiments, the method further comprises repairing said cleaved target polynucleotide by homologous recombination with an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide. In some embodiments, said mutation results in one or more amino acid changes in a protein expression from a gene comprising the target sequence.

[0019] In one aspect, the invention provides a method for developing a biologically active agent that modulates a cell signaling event associated with a disease gene. In some embodiments, a disease gene is any gene associated with an increase in the risk of having or developing a disease. In some embodiments, the method comprises (a) contacting a test compound with a model cell of any one of the described embodiments; and (b) detecting a change in a readout that is indicative of a reduction or an augmentation of a cell signaling event associated with said mutation in said disease gene, thereby developing said biologically active agent that modulates said cell signaling event associated with said disease gene.

[0020] In one aspect, the invention provides a recombinant polynucleotide comprising a guide sequence upstream of a tracr mate sequence, wherein the guide sequence when expressed directs sequence-specific binding of a CRISPR complex to a corresponding target sequence present in a eukaryotic cell. In some embodiments, the target sequence is a viral sequence present in a eukaryotic cell. In some embodiments, the target sequence is a proto-oncogene or an oncogene.

[0021] In one aspect the invention provides for a method of selecting one or more prokaryotic cell(s) by introducing one or more mutations in a gene in the one or more prokaryotic cell (s), the method comprising: introducing one or more vectors into the prokaryotic cell (s), wherein the one or more vectors drive expression of one or more of: a CRISPR enzyme, a guide sequence linked to a tracr mate sequence, a tracr sequence, and an editing template; wherein the editing template comprises the one or more mutations that abolish CRISPR enzyme cleavage; allowing homologous recombination

nation of the editing template with the target polynucleotide in the cell(s) to be selected; allowing a CRISPR complex to bind to a target polynucleotide to effect cleavage of the target polynucleotide within said gene, wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence within the target polynucleotide, and (2) the tracr mate sequence that is hybridized to the tracr sequence, wherein binding of the CRISPR complex to the target polynucleotide induces cell death, thereby allowing one or more prokaryotic cell(s) in which one or more mutations have been introduced to be selected. In a preferred embodiment, the CRISPR enzyme is Cas9. In another aspect of the invention the cell to be selected may be a eukaryotic cell. Aspects of the invention allow for selection of specific cells without requiring a selection marker or a two-step process that may include a counter-selection system.

[0022] In some aspects the invention provides a non-naturally occurring or engineered composition comprising a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises (a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell, (b) a tracr mate sequence, and (c) a tracr sequence wherein (a), (b) and (c) are arranged in a 5' to 3' orientation, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, wherein the CRISPR complex comprises a CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence,

[0023] or

[0024] a CRISPR enzyme system, wherein the system is encoded by a vector system comprising one or more vectors comprising I. a first regulatory element operably linked to a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises (a) one or more guide sequences capable of hybridizing to one or more target sequences in a eukaryotic cell, (b) a tracr mate sequence, and (c) one or more tracr sequences, and II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising at least one or more nuclear localization sequences, wherein (a), (b) and (c) are arranged in a 5' to 3' orientation, wherein components I and II are located on the same or different vectors of the system, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence, or a multiplexed CRISPR enzyme system, wherein the system is encoded by a vector system comprising one or more vectors comprising I. a first regulatory element operably linked to (a) one or more guide sequences capable of hybridizing to a target sequence in a cell, and (b) at least one or more tracr mate sequences, II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and III. a third regulatory element operably linked to a tracr sequence, wherein components I, II and III are located on the same or different vectors of the system, wherein when transcribed, the tracr mate sequence hybridizes

to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence, and wherein in the multiplexed system multiple guide sequences and a single tracr sequence is used; and wherein one or more of the guide, tracr and tracr mate sequences are modified to improve stability.

[0025] In aspects of the invention, the modification comprises an engineered secondary structure. For example, the modification can comprise a reduction in a region of hybridization between the tracr mate sequence and the tracr sequence. For example, the modification also may comprise fusing the tracr mate sequence and the tracr sequence through an artificial loop. The modification may comprise the tracr sequence having a length between 40 and 120 bp. In embodiments of the invention, the tracr sequence is between 40 bp and full length of the tracr. In certain embodiments, the length of tracrRNA includes at least nucleotides 1-67 and in some embodiments at least nucleotides 1-85 of the wild type tracrRNA. In some embodiments, at least nucleotides corresponding to nucleotides 1-67 or 1-85 of wild type *S. pyogenes* Cas9 tracrRNA may be used. Where the CRISPR system uses enzymes other than Cas9, or other than SpCas9, then corresponding nucleotides in the relevant wild type tracrRNA may be present. In some embodiments, the length of tracrRNA includes no more than nucleotides 1-67 or 1-85 of the wild type tracrRNA. The modification may comprise sequence optimization. In certain aspects, sequence optimization may comprise reducing the incidence of polyT sequences in the tracr and/or tracr mate sequence. Sequence optimization may be combined with reduction in the region of hybridization between the tracr mate sequence and the tracr sequence; for example, a reduced length tracr sequence.

[0026] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises reduction in polyT sequences in the tracr and/or tracr mate sequence. In some aspects of the invention, one or more Ts present in a poly-T sequence of the relevant wild type sequence (that is, a stretch of more than 3, 4, 5, 6, or more contiguous T bases; in some embodiments, a stretch of no more than 10, 9, 8, 7, 6 contiguous T bases) may be substituted with a non-T nucleotide, e.g., an A, so that the string is broken down into smaller stretches of Ts with each stretch having 4, or fewer than 4 (for example, 3 or 2) contiguous Ts. Bases other than A may be used for substitution, for example C or G, or non-naturally occurring nucleotides or modified nucleotides. If the string of Ts is involved in the formation of a hairpin (or stem loop), then it is advantageous that the complementary base for the non-T base be changed to complement the non-T nucleotide. For example, if the non-T base is an A, then its complement may be changed to a T, e.g., to preserve or assist in the preservation of secondary structure. For instance, 5'-TTTTT can be altered to become 5'-TT-TAT and the complementary 5'-AAAAA can be changed into 5'-ATAAA.

[0027] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises adding a polyT terminator sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises adding a polyT terminator sequence in tracr and/or tracr mate

sequences. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises adding a polyT terminator sequence in the guide sequence. The polyT terminator sequence may comprise 5 contiguous T bases, or more than 5.

[0028] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises altering loops and/or hairpins. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing a minimum of two hairpins in the guide sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing a hairpin formed by complementation between the tracr and tracr mate (direct repeat) sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing one or more further hairpin(s) at or towards the 3' end of the tracrRNA sequence. For example, a hairpin may be formed by providing self complementary sequences within the tracrRNA sequence joined by a loop such that a hairpin is formed on self folding. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing additional hairpins added to the 3' of the guide sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises extending the 5' end of the guide sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing one or more hairpins in the 5' end of the guide sequence. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises appending the sequence (5'-AGGACGAAGTCCTAA) to the 5' end of the guide sequence. Other sequences suitable for forming hairpins will be known to the skilled person, and may be used in certain aspects of the invention. In some aspects of the invention, at least 2, 3, 4, 5, or more additional hairpins are provided. In some aspects of the invention, no more than 10, 9, 8, 7, 6 additional hairpins are provided. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises two hairpins. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises three hairpins. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises at most five hairpins.

[0029] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises providing cross linking, or providing one or more modified nucleotides in the polynucleotide sequence. Modified nucleotides and/or cross linking may be provided in any or all of the tracr, tracr mate, and/or guide sequences, and/or in the enzyme coding sequence, and/or in vector sequences. Modifications may include inclusion of at least one non naturally occurring nucleotide, or a modified nucleotide, or analogs thereof. Modified nucleotides may be modified at the ribose, phosphate, and/or base moiety. Modified nucleotides may include 2'-O-methyl analogs, 2'-deoxy analogs, or 2'-fluoro analogs. The nucleic acid backbone may be modified, for example, a phosphorothioate backbone may be used. The use of locked nucleic acids (LNA) or bridged nucleic acids (BNA) may also be possible. Further examples of modi-

fied bases include, but are not limited to, 2-aminopurine, 5-bromo-uridine, pseudouridine, inosine, 7-methylguanosine.

[0030] It will be understood that any or all of the above modifications may be provided in isolation or in combination in a given CRISPR-Cas system or CRISPR enzyme system. Such a system may include one, two, three, four, five, or more of said modifications.

[0031] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the CRISPR enzyme is a type II CRISPR system enzyme, e.g., a Cas9 enzyme. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the CRISPR enzyme is comprised of less than one thousand amino acids, or less than four thousand amino acids. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the Cas9 enzyme is StCas9 or St1Cas9, or the Cas9 enzyme is a Cas9 enzyme from an organism selected from the group consisting of genus *Streptococcus*, *Campylobacter*, *Nitratifactor*, *Staphylococcus*, *Parvibaculum*, *Roseburia*, *Neisseria*, *Gluconacetobacter*, *Azospirillum*, *Sphaerochaeta*, *Lactobacillus*, *Eubacterium* or *Corynebacter*. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the CRISPR enzyme is a nuclease directing cleavage of both strands at the location of the target sequence.

[0032] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the first regulatory element is a polymerase III promoter. In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the second regulatory element is a polymerase II promoter.

[0033] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the guide sequence comprises at least fifteen nucleotides.

[0034] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the modification comprises optimized tracr sequence and/or optimized guide sequence RNA and/or co-fold structure of tracr sequence and/or tracr mate sequence(s) and/or stabilizing secondary structures of tracr sequence and/or tracr sequence with a reduced region of base-pairing and/or tracr sequence fused RNA elements; and/or, in the multiplexed system there are two RNAs comprising a tracer and comprising a plurality of guides or one RNA comprising a plurality of chimerics.

[0035] In aspects of the invention the chimeric RNA architecture is further optimized according to the results of mutagenesis studies. In chimeric RNA with two or more hairpins, mutations in the proximal direct repeat to stabilize the hairpin may result in ablation of CRISPR complex activity. Mutations in the distal direct repeat to shorten or stabilize the hairpin may have no effect on CRISPR complex activity. Sequence randomization in the bulge region between the proximal and distal repeats may significantly reduce CRISPR complex activity. Single base pair changes or sequence randomization in the linker region between hairpins may result in complete loss of CRISPR complex activity. Hairpin stabilization of the distal hairpins that follow the first hairpin after the guide sequence may result in maintenance or improvement of CRISPR complex activity. Accordingly, in preferred embodiments of the invention, the chimeric RNA architecture may be further optimized by generating a smaller chimeric RNA which may be beneficial for therapeutic delivery options and other uses and this may be achieved by altering

the distal direct repeat so as to shorten or stabilize the hairpin. In further preferred embodiments of the invention, the chimeric RNA architecture may be further optimized by stabilizing one or more of the distal hairpins. Stabilization of hairpins may include modifying sequences suitable for forming hairpins. In some aspects of the invention, at least 2, 3, 4, 5, or more additional hairpins are provided. In some aspects of the invention, no more than 10, 9, 8, 7, 6 additional hairpins are provided. In some aspects of the invention stabilization may be cross linking and other modifications. Modifications may include inclusion of at least one non naturally occurring nucleotide, or a modified nucleotide, or analogs thereof. Modified nucleotides may be modified at the ribose, phosphate, and/or base moiety. Modified nucleotides may include 2'-O-methyl analogs, 2'-deoxy analogs, or 2'-fluoro analogs. The nucleic acid backbone may be modified, for example, a phosphorothioate backbone may be used. The use of locked nucleic acids (LNA) or bridged nucleic acids (BNA) may also be possible. Further examples of modified bases include, but are not limited to, 2-aminopurine, 5-bromo-uridine, pseudouridine, inosine, 7-methylguanosine.

[0036] In an aspect the invention provides the CRISPR-Cas system or CRISPR enzyme system wherein the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell.

[0037] Accordingly, in some aspects of the invention, the length of tracrRNA required in a construct of the invention, e.g., a chimeric construct, need not necessarily be fixed, and in some aspects of the invention it can be between 40 and 120 bp, and in some aspects of the invention up to the full length of the tracr, e.g., in some aspects of the invention, until the 3' end of tracr as punctuated by the transcription termination signal in the bacterial genome. In certain embodiments, the length of tracrRNA includes at least nucleotides 1-67 and in some embodiments at least nucleotides 1-85 of the wild type tracrRNA. In some embodiments, at least nucleotides corresponding to nucleotides 1-67 or 1-85 of wild type *S. pyogenes* Cas9 tracrRNA may be used. Where the CRISPR system uses enzymes other than Cas9, or other than SpCas9, then corresponding nucleotides in the relevant wild type tracrRNA may be present. In some embodiments, the length of tracrRNA includes no more than nucleotides 1-67 or 1-85 of the wild type tracrRNA. With respect to sequence optimization (e.g., reduction in polyT sequences), e.g., as to strings of Ts internal to the tracr mate (direct repeat) or tracrRNA, in some aspects of the invention, one or more Ts present in a poly-T sequence of the relevant wild type sequence (that is, a stretch of more than 3, 4, 5, 6, or more contiguous T bases; in some embodiments, a stretch of no more than 10, 9, 8, 7, 6 contiguous T bases) may be substituted with a non-T nucleotide, e.g., an A, so that the string is broken down into smaller stretches of Ts with each stretch having 4, or fewer than 4 (for example, 3 or 2) contiguous Ts. If the string of Ts is involved in the formation of a hairpin (or stem loop), then it is advantageous that the complementary base for the non-T base be changed to complement the non-T nucleotide. For example, if the non-T base is an A, then its complement may be changed to a T, e.g., to preserve or assist in the preservation of secondary structure. For instance, 5'-TTTTT can be altered to become 5'-TTTAT and the complementary 5'-AAAAA can be changed into 5'-ATAAA. As to the presence of polyT terminator sequences in tracr+tracr mate transcript, e.g., a polyT terminator (TTTTT or more), in some aspects of the invention it is advantageous that such be added to end of the transcript,

whether it is in two RNA (tracr and tracr mate) or single guide RNA form. Concerning loops and hairpins in tracr and tracr mate transcripts, in some aspects of the invention it is advantageous that a minimum of two hairpins be present in the chimeric guide RNA. A first hairpin can be the hairpin formed by complementation between the tracr and tracr mate (direct repeat) sequence. A second hairpin can be at the 3' end of the tracrRNA sequence, and this can provide secondary structure for interaction with Cas9. Additional hairpins may be added to the 3' of the guide RNA, e.g., in some aspects of the invention to increase the stability of the guide RNA. Additionally, the 5' end of the guide RNA, in some aspects of the invention, may be extended. In some aspects of the invention, one may consider 20 bp in the 5' end as a guide sequence. The 5' portion may be extended. One or more hairpins can be provided in the 5' portion, e.g., in some aspects of the invention, this may also improve the stability of the guide RNA. In some aspects of the invention, the specific hairpin can be provided by appending the sequence (5'-AGGACGAAGTCCTAA) to the 5' end of the guide sequence, and, in some aspects of the invention, this may help improve stability. Other sequences suitable for forming hairpins will be known to the skilled person, and may be used in certain aspects of the invention. In some aspects of the invention, at least 2, 3, 4, 5, or more additional hairpins are provided. In some aspects of the invention, no more than 10, 9, 8, 7, 6 additional hairpins are provided. The foregoing also provides aspects of the invention involving secondary structure in guide sequences. In some aspects of the invention there may be cross linking and other modifications, e.g., to improve stability. Modifications may include inclusion of at least one non naturally occurring nucleotide, or a modified nucleotide, or analogs thereof. Modified nucleotides may be modified at the ribose, phosphate, and/or base moiety. Modified nucleotides may include 2'-O-methyl analogs, 2'-deoxy analogs, or 2'-fluoro analogs. The nucleic acid backbone may be modified, for example, a phosphorothioate backbone may be used. The use of locked nucleic acids (LNA) or bridged nucleic acids (BNA) may also be possible. Further examples of modified bases include, but are not limited to, 2-aminopurine, 5-bromo-uridine, pseudouridine, inosine, 7-methylguanosine. Such modifications or cross linking may be present in the guide sequence or other sequences adjacent the guide sequence.

[0038] Accordingly, it is an object of the invention not to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0039] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprising", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essen-

tially of” have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention. These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0041] FIG. 1 shows a schematic model of the CRISPR system. The Cas9 nuclease from *Streptococcus pyogenes* (yellow) is targeted to genomic DNA by a synthetic guide RNA (sgRNA) consisting of a 20-nt guide sequence (blue) and a scaffold (red). The guide sequence base-pairs with the DNA target (blue), directly upstream of a requisite 5'-NGG protospacer adjacent motif (PAM; magenta), and Cas9 mediates a double-stranded break (DSB) ~3 bp upstream of the PAM (red triangle).

[0042] FIG. 2A-F illustrates an exemplary CRISPR system, a possible mechanism of action, an example adaptation for expression in eukaryotic cells, and results of tests assessing nuclear localization and CRISPR activity.

[0043] FIG. 3A-C illustrates an exemplary expression cassette for expression of CRISPR system elements in eukaryotic cells, predicted structures of example guide sequences, and CRISPR system activity as measured in eukaryotic and prokaryotic cells.

[0044] FIG. 4A-D illustrates results of an evaluation of SpCas9 specificity for an example target.

[0045] FIG. 5A-G illustrates an exemplary vector system and results for its use in directing homologous recombination in eukaryotic cells.

[0046] FIG. 6A-C illustrates a comparison of different tracrRNA transcripts for Cas9-mediated gene targeting.

[0047] FIG. 7A-D illustrates an exemplary CRISPR system, an example adaptation for expression in eukaryotic cells, and results of tests assessing CRISPR activity.

[0048] FIG. 8A-C illustrates exemplary manipulations of a CRISPR system for targeting of genomic loci in mammalian cells.

[0049] FIG. 9A-B illustrates the results of a Northern blot analysis of crRNA processing in mammalian cells.

[0050] FIG. 10A-C illustrates a schematic representation of chimeric RNAs and results of SURVEYOR assays for CRISPR system activity in eukaryotic cells.

[0051] FIG. 11A-B illustrates a graphical representation of the results of SURVEYOR assays for CRISPR system activity in eukaryotic cells.

[0052] FIG. 12 illustrates predicted secondary structures for exemplary chimeric RNAs comprising a guide sequence, tracr mate sequence, and tracr sequence.

[0053] FIG. 13A-D is a phylogenetic tree of Cas genes

[0054] FIG. 14A-F shows the phylogenetic analysis revealing five families of Cas9s, including three groups of large Cas9s (~1400 amino acids) and two of small Cas9s (~1100 amino acids).

[0055] FIG. 15 shows a graph depicting the function of different optimized guide RNAs.

[0056] FIG. 16 shows the sequence and structure of different guide chimeric RNAs.

[0057] FIG. 17 shows the co-fold structure of the tracrRNA and direct repeat.

[0058] FIGS. 18 A and B shows data from the StlCas9 chimeric guide RNA optimization in vitro.

[0059] FIG. 19A-B shows cleavage of either unmethylated or methylated targets by SpCas9 cell lysate.

[0060] FIG. 20A-G shows the optimization of guide RNA architecture for SpCas9-mediated mammalian genome editing. (a) Schematic of bicistronic expression vector (PX330) for U6 promoter-driven single guide RNA (sgRNA) and CBh promoter-driven human codon-optimized *Streptococcus pyogenes* Cas9 (hSpCas9) used for all subsequent experiments. The sgRNA consists of a 20-nt guide sequence (blue) and scaffold (red), truncated at various positions as indicated. (b) SURVEYOR assay for SpCas9-mediated indels at the human EMX1 and PVALB loci. Arrows indicate the expected SURVEYOR fragments (n=3). (c) Northern blot analysis for the four sgRNA truncation architectures, with U1 as loading control. (d) Both wildtype (wt) or nickase mutant (D10A) of SpCas9 promoted insertion of a HindIII site into the human EMX1 gene. Single stranded oligonucleotides (ssODNs), oriented in either the sense or antisense direction relative to genome sequence, were used as homologous recombination templates. (e) Schematic of the human SERPINB5 locus. sgRNAs and PAMs are indicated by colored bars above sequence; methylcytosine (Me) are highlighted (pink) and numbered relative to the transcriptional start site (TSS, +1). (f) Methylation status of SERPINB5 assayed by bisulfite sequencing of 16 clones. Filled circles, methylated CpG; open circles, unmethylated CpG. (g) Modification efficiency by three sgRNAs targeting the methylated region of SERPINB5, assayed by deep sequencing (n=2). Error bars indicate Wilson intervals (Online Methods).

[0061] FIG. 21A-B shows the further optimization of CRISPR-Cas sgRNA architecture. (a) Schematic of four additional sgRNA architectures, I-IV. Each consists of a 20-nt guide sequence (blue) joined to the direct repeat (DR, grey), which hybridizes to the tracrRNA (red). The DR-tracrRNA hybrid is truncated at +12 or +22, as indicated, with an artificial GAAA stem loop. tracrRNA truncation positions are numbered according to the previously reported transcription start site for tracrRNA. sgRNA architectures II and IV carry mutations within their poly-U tracts, which could serve as premature transcriptional terminators. (b) SURVEYOR assay for SpCas9-mediated indels at the human EMX1 locus for target sites 1-3. Arrows indicate the expected SURVEYOR fragments (n=3).

[0062] FIG. 22 illustrates visualization of some target sites in the human genome.

[0063] FIG. 23A-B shows (A) a schematic of the sgRNA and (B) the SURVEYOR analysis of five sgRNA variants for SaCas9 for an optimal truncated architecture with highest cleavage efficiency

[0064] The figures herein are for illustrative purposes only and are not necessarily drawn to scale.

DETAILED DESCRIPTION OF THE INVENTION

[0065] The terms “polynucleotide”, “nucleotide”, “nucleotide sequence”, “nucleic acid” and “oligonucleotide” are used interchangeably. They refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may

have any three dimensional structure, and may perform any function, known or unknown. The following are non limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, short interfering RNA (siRNA), short-hairpin RNA (shRNA), micro-RNA (miRNA), ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise one or more modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[0066] In aspects of the invention the terms “chimeric RNA”, “chimeric guide RNA”, “guide RNA”, “single guide RNA” and “synthetic guide RNA” are used interchangeably and refer to the polynucleotide sequence comprising the guide sequence, the tracr sequence and the tracr mate sequence. The term “guide sequence” refers to the about 20 bp sequence within the guide RNA that specifies the target site and may be used interchangeably with the terms “guide” or “spacer”. The term “tracr mate sequence” may also be used interchangeably with the term “direct repeat(s)”.

[0067] As used herein the term “wild type” is a term of the art understood by skilled persons and means the typical form of an organism, strain, gene or characteristic as it occurs in nature as distinguished from mutant or variant forms.

[0068] As used herein the term “variant” should be taken to mean the exhibition of qualities that have a pattern that deviates from what occurs in nature.

[0069] The terms “non-naturally occurring” or “engineered” are used interchangeably and indicate the involvement of the hand of man. The terms, when referring to nucleic acid molecules or polypeptides mean that the nucleic acid molecule or the polypeptide is at least substantially free from at least one other component with which they are naturally associated in nature and as found in nature.

[0070] “Complementarity” refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base-pairing or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). “Perfectly complementary” means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. “Substantially complementary” as used herein refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions.

[0071] As used herein, “stringent conditions” for hybridization refer to conditions under which a nucleic acid having complementarity to a target sequence predominantly hybridizes with the target sequence, and substantially does not

hybridize to non-target sequences. Stringent conditions are generally sequence-dependent, and vary depending on a number of factors. In general, the longer the sequence, the higher the temperature at which the sequence specifically hybridizes to its target sequence. Non-limiting examples of stringent conditions are described in detail in Tijssen (1993), *Laboratory Techniques In Biochemistry And Molecular Biology-Hybridization With Nucleic Acid Probes Part I*, Second Chapter “Overview of principles of hybridization and the strategy of nucleic acid probe assay”, Elsevier, N.Y.

[0072] “Hybridization” refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson Crick base pairing, Hoogsteen binding, or in any other sequence specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi stranded complex, a single self hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of PCR, or the cleavage of a polynucleotide by an enzyme. A sequence capable of hybridizing with a given sequence is referred to as the “complement” of the given sequence.

[0073] As used herein, “stabilization” or “increasing stability” with respect to components of the CRISPR system relate to securing or steadying the structure of the molecule. This may be accomplished by introduction of one or mutations, including single or multiple base pair changes, increasing the number of hair pins, cross linking, breaking up particular stretches of nucleotides and other modifications. Modifications may include inclusion of at least one non naturally occurring nucleotide, or a modified nucleotide, or analogs thereof. Modified nucleotides may be modified at the ribose, phosphate, and/or base moiety. Modified nucleotides may include 2'-O-methyl analogs, 2'-deoxy analogs, or 2'-fluoro analogs. The nucleic acid backbone may be modified, for example, a phosphorothioate backbone may be used. The use of locked nucleic acids (LNA) or bridged nucleic acids (BNA) may also be possible. Further examples of modified bases include, but are not limited to, 2-aminopurine, 5-bromo-uridine, pseudouridine, inosine, 7-methylguanosine. These modifications may apply to any component of the CRISPR system. In a preferred embodiment these modifications are made to the RNA components, e.g. the guide RNA or chimeric polynucleotide sequence.

[0074] As used herein, “expression” refers to the process by which a polynucleotide is transcribed from a DNA template (such as into mRNA or other RNA transcript) and/or the process by which a transcribed mRNA is subsequently translated into peptides, polypeptides, or proteins. Transcripts and encoded polypeptides may be collectively referred to as “gene product.” If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[0075] The terms “polypeptide”, “peptide” and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component. As used herein the

term “amino acid” includes natural and/or unnatural or synthetic amino acids, including glycine and both the D or L optical isomers, and amino acid analogs and peptidomimetics.

[0076] The terms “subject,” “individual,” and “patient” are used interchangeably herein to refer to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, murines, simians, humans, farm animals, sport animals, and pets. Tissues, cells and their progeny of a biological entity obtained in vivo or cultured in vitro are also encompassed. In some embodiments, a subject may be an invertebrate animal, for example, an insect or a nematode; while in others, a subject may be a plant or a fungus.

[0077] The terms “therapeutic agent,” “therapeutic capable agent” or “treatment agent” are used interchangeably and refer to a molecule or compound that confers some beneficial effect upon administration to a subject. The beneficial effect includes enablement of diagnostic determinations; amelioration of a disease, symptom, disorder, or pathological condition; reducing or preventing the onset of a disease, symptom, disorder or condition; and generally counteracting a disease, symptom, disorder or pathological condition.

[0078] As used herein, “treatment” or “treating,” or “palliating” or “ameliorating” are used interchangeably. These terms refer to an approach for obtaining beneficial or desired results including but not limited to a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant any therapeutically relevant improvement in or effect on one or more diseases, conditions, or symptoms under treatment. For prophylactic benefit, the compositions may be administered to a subject at risk of developing a particular disease, condition, or symptom, or to a subject reporting one or more of the physiological symptoms of a disease, even though the disease, condition, or symptom may not have yet been manifested.

[0079] The term “effective amount” or “therapeutically effective amount” refers to the amount of an agent that is sufficient to effect beneficial or desired results. The therapeutically effective amount may vary depending upon one or more of: the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art. The term also applies to a dose that will provide an image for detection by any one of the imaging methods described herein. The specific dose may vary depending on one or more of: the particular agent chosen, the dosing regimen to be followed, whether it is administered in combination with other compounds, timing of administration, the tissue to be imaged, and the physical delivery system in which it is carried.

[0080] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. See Sambrook, Fritsch and Maniatis, *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd edition (1989); *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY* (F. M. Ausubel, et al. eds., (1987)); the series *METHODS IN ENZYMOLOGY* (Academic Press, Inc.): *PCR 2: A PRACTICAL APPROACH* (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *ANTIBODIES*, A

LABORATORY MANUAL, and *ANIMAL CELL CULTURE* (R. I. Freshney, ed. (1987)).

[0081] Several aspects of the invention relate to vector systems comprising one or more vectors, or vectors as such. Vectors can be designed for expression of CRISPR transcripts (e.g. nucleic acid transcripts, proteins, or enzymes) in prokaryotic or eukaryotic cells. For example, CRISPR transcripts can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells. Suitable host cells are discussed further in Goeddel, *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

[0082] Vectors may be introduced and propagated in a prokaryote. In some embodiments, a prokaryote is used to amplify copies of a vector to be introduced into a eukaryotic cell or as an intermediate vector in the production of a vector to be introduced into a eukaryotic cell (e.g. amplifying a plasmid as part of a viral vector packaging system). In some embodiments, a prokaryote is used to amplify copies of a vector and express one or more nucleic acids, such as to provide a source of one or more proteins for delivery to a host cell or host organism. Expression of proteins in prokaryotes is most often carried out in *Escherichia coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, such as to the amino terminus of the recombinant protein. Such fusion vectors may serve one or more purposes, such as: (i) to increase expression of recombinant protein; (ii) to increase the solubility of the recombinant protein; and (iii) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Example fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

[0083] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann et al., (1988) *Gene* 69:301-315) and pET 11d (Studier et al., *GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY* 185, Academic Press, San Diego, Calif. (1990) 60-89).

[0084] In some embodiments, a vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, et al., 1987. *EMBO J.* 6: 229-234), pMFa (Kuijan and Herskowitz, 1982. *Cell* 30: 933-943), pJRY88 (Schultz et al., 1987. *Gene* 54: 113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

[0085] In some embodiments, a vector drives protein expression in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., SF9 cells) include the pAc series

(Smith, et al., 1983. *Mol. Cell. Biol.* 3: 2156-2165) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

[0086] In some embodiments, a vector is capable of driving expression of one or more sequences in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, 1987. *Nature* 329: 840) and pMT2PC (Kaufman, et al., 1987. *EMBO J.* 6: 187-195). When used in mammalian cells, the expression vector's control functions are typically provided by one or more regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, simian virus 40, and others disclosed herein and known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells see, e.g., Chapters 16 and 17 of Sambrook, et al., *MOLECULAR CLONING: A LABORATORY MANUAL*. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[0087] In some embodiments, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, et al., 1987. *Genes Dev.* 1: 268-277), lymphoid-specific promoters (Calame and Eaton, 1988. *Adv. Immunol.* 43: 235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989. *EMBO J.* 8: 729-733) and immunoglobulins (Baneiji, et al., 1983. *Cell* 33: 729-740; Queen and Baltimore, 1983. *Cell* 33: 741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, 1989. *Proc. Natl. Acad. Sci. USA* 86: 5473-5477), pancreas-specific promoters (Edlund, et al., 1985. *Science* 230: 912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, e.g., the murine hox promoters (Kessel and Gruss, 1990. *Science* 249: 374-379) and the α -fetoprotein promoter (Campes and Tilghman, 1989. *Genes Dev.* 3: 537-546).

[0088] In some embodiments, a regulatory element is operably linked to one or more elements of a CRISPR system so as to drive expression of the one or more elements of the CRISPR system. In general, CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats), also known as SPIDRs (SPacer Interspersed Direct Repeats), constitute a family of DNA loci that are usually specific to a particular bacterial species. The CRISPR locus comprises a distinct class of interspersed short sequence repeats (SSRs) that were recognized in *E. coli* (Ishino et al., *J. Bacteriol.*, 169:5429-5433 [1987]; and Nakata et al., *J. Bacteriol.*, 171:3553-3556 [1989]), and associated genes. Similar interspersed SSRs have been identified in *Haloferax mediterranei*, *Streptococcus pyogenes*, *Anabaena*, and *Mycobacterium tuberculosis* (See, Groenen et al., *Mol. Microbiol.*, 10:1057-1065 [1993]; Hoe et al., *Emerg. Infect. Dis.*, 5:254-263 [1999]; Masepohl et al., *Biochim. Biophys. Acta* 1307:26-30 [1996]; and Mojica et al., *Mol. Microbiol.*, 17:85-93 [1995]). The CRISPR loci typically differ from other SSRs by the structure of the repeats, which have been termed short regularly spaced repeats (SRSRs) (Janssen et al., *OMICS J. Integ. Biol.*, 6:23-33 [2002]; and Mojica et al., *Mol. Microbiol.*, 36:244-246

[2000]). In general, the repeats are short elements that occur in clusters that are regularly spaced by unique intervening sequences with a substantially constant length (Mojica et al., [2000], *supra*). Although the repeat sequences are highly conserved between strains, the number of interspersed repeats and the sequences of the spacer regions typically differ from strain to strain (van Embden et al., *J. Bacteriol.*, 182:2393-2401 [2000]). CRISPR loci have been identified in more than 40 prokaryotes (See e.g., Jansen et al., *Mol. Microbiol.*, 43:1565-1575 [2002]; and Mojica et al., [2005]) including, but not limited to *Aeropyrum*, *Pyrobaculum*, *Sulfolobus*, *Archaeoglobus*, *Halocarcula*, *Methanobacterium*, *Methanococcus*, *Methanosarcina*, *Methanopyrus*, *Pyrococcus*, *Picrophilus*, *Thermoplasma*, *Corynebacterium*, *Mycobacterium*, *Streptomyces*, *Aquifex*, *Porphyromonas*, *Chlorobium*, *Thermus*, *Bacillus*, *Listeria*, *Staphylococcus*, *Clostridium*, *Thermotoga*, *Mycoplasmata*, *Fusobacterium*, *Azarcus*, *Chromobacterium*, *Neisseria*, *Nitrosomonas*, *Desulfovibrio*, *Geobacter*, *Myxococcus*, *Campylobacter*, *Wolinella*, *Acinetobacter*, *Erwinia*, *Escherichia*, *Legionella*, *Methylococcus*, *Pasteurella*, *Photobacterium*, *Salmonella*, *Xanthomonas*, *Yersinia*, *Treponema*, and *Thermotoga*.

[0089] In general, "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (transactivating CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus. In some embodiments, one or more elements of a CRISPR system is derived from a type I, type II, or type III CRISPR system. In some embodiments, one or more elements of a CRISPR system is derived from a particular organism comprising an endogenous CRISPR system, such as *Streptococcus pyogenes*. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, "target sequence" refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a target sequence and a guide sequence promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridisation and promote formation of a CRISPR complex. A target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. In some embodiments, a target sequence is located in the nucleus or cytoplasm of a cell. In some embodiments, the target sequence may be within an organelle of a eukaryotic cell, for example, mitochondrion or chloroplast. A sequence or template that may be used for recombination into the targeted locus comprising the target sequences is referred to as an "editing template" or "editing polynucleotide" or "editing sequence". In aspects of the invention, an exogenous template polynucleotide may be referred to as an editing template. In an aspect of the invention the recombination is homologous recombination.

[0090] Typically, in the context of an endogenous CRISPR system, formation of a CRISPR complex (comprising a guide sequence hybridized to a target sequence and complexed with

one or more Cas proteins) results in cleavage of one or both strands in or near (e.g. within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of a CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence. In some embodiments, the tracr sequence has sufficient complementarity to a tracr mate sequence to hybridize and participate in formation of a CRISPR complex. As with the target sequence, it is believed that complete complementarity is not needed, provided there is sufficient to be functional. In some embodiments, the tracr sequence has at least 50%, 60%, 70%, 80%, 90%, 95% or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned. In some embodiments, one or more vectors driving expression of one or more elements of a CRISPR system are introduced into a host cell such that expression of the elements of the CRISPR system direct formation of a CRISPR complex at one or more target sites. For example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. CRISPR system elements that are combined in a single vector may be arranged in any suitable orientation, such as one element located 5' with respect to ("upstream" of) or 3' with respect to ("downstream" of) a second element. The coding sequence of one element may be located on the same or opposite strand of the coding sequence of a second element, and oriented in the same or opposite direction. In some embodiments, a single promoter drives expression of a transcript encoding a CRISPR enzyme and one or more of the guide sequence, tracr mate sequence (optionally operably linked to the guide sequence), and a tracr sequence embedded within one or more intron sequences (e.g. each in a different intron, two or more in at least one intron, or all in a single intron). In some embodiments, the CRISPR enzyme, guide sequence, tracr mate sequence, and tracr sequence are operably linked to and expressed from the same promoter.

[0091] In some embodiments, a vector comprises one or more insertion sites, such as a restriction endonuclease recognition sequence (also referred to as a "cloning site"). In some embodiments, one or more insertion sites (e.g. about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more insertion sites) are located upstream and/or downstream of one or more sequence elements of one or more vectors. In some embodiments, a vector comprises an insertion site upstream of a tracr mate sequence, and optionally downstream of a regulatory element operably linked to the tracr mate sequence, such that following insertion of a guide sequence into the insertion site and upon expression the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a eukaryotic cell. In some embodiments, a vector comprises two or more insertion sites, each insertion site being located between two tracr mate sequences so as to allow insertion of a guide sequence at each site. In such an arrangement, the two

or more guide sequences may comprise two or more copies of a single guide sequence, two or more different guide sequences, or combinations of these. When multiple different guide sequences are used, a single expression construct may be used to target CRISPR activity to multiple different, corresponding target sequences within a cell. For example, a single vector may comprise about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or more guide sequences. In some embodiments, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more such guide-sequence-containing vectors may be provided, and optionally delivered to a cell.

[0092] In some embodiments, a vector comprises a regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, such as a Cas protein. Non-limiting examples of Cas proteins include Cas1, CasB, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, homologs thereof, or modified versions thereof. These enzymes are known; for example, the amino acid sequence of *S. pyogenes* Cas9 protein may be found in the SwissProt database under accession number Q99ZW2. In some embodiments, the unmodified CRISPR enzyme has DNA cleavage activity, such as Cas9. In some embodiments the CRISPR enzyme is Cas9, and may be Cas9 from *S. pyogenes* or *S. pneumoniae*. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. In some embodiments, the CRISPR enzyme directs cleavage of one or both strands within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 50, 100, 200, 500, or more base pairs from the first or last nucleotide of a target sequence. In some embodiments, a vector encodes a CRISPR enzyme that is mutated to with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). Other examples of mutations that render Cas9 a nickase include, without limitation, H840A, N854A, and N863A. In some embodiments, a Cas9 nickase may be used in combination with guide sequence(s), e.g., two guide sequences, which target respectively sense and antisense strands of the DNA target. This combination allows both strands to be nicked and used to induce NHEJ. Applicants have demonstrated (data not shown) the efficacy of two nickase targets (i.e., sgRNAs targeted at the same location but to different strands of DNA) in inducing mutagenic NHEJ. A single nickase (Cas9-D10A with a single sgRNA) is unable to induce NHEJ and create indels but Applicants have shown that double nickase (Cas9-D10A and two sgRNAs targeted to different strands at the same location) can do so in human embryonic stem cells (hESCs). The efficiency is about 50% of nuclease (i.e., regular Cas9 without D10 mutation) in hESCs.

[0093] As a further example, two or more catalytic domains of Cas9 (RuvC I, RuvC II, and RuvC III) may be mutated to produce a mutated Cas9 substantially lacking all DNA cleavage activity. In some embodiments, a D10A mutation is combined with one or more of H840A, N854A, or N863A muta-

tions to produce a Cas9 enzyme substantially lacking all DNA cleavage activity. In some embodiments, a CRISPR enzyme is considered to substantially lack all DNA cleavage activity when the DNA cleavage activity of the mutated enzyme is less than about 25%, 10%, 5%, 1%, 0.1%, 0.01%, or lower with respect to its non-mutated form. Other mutations may be useful; where the Cas9 or other CRISPR enzyme is from a species other than *S. pyogenes*, mutations in corresponding amino acids may be made to achieve similar effects.

[0094] In some embodiments, an enzyme coding sequence encoding a CRISPR enzyme is codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, mouse, rat, rabbit, dog, or non-human primate. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon (e.g. about or more than about 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization. Codon usage tables are readily available, for example, at the “Codon Usage Database”, and these tables can be adapted in a number of ways. See Nakamura, Y., et al. “Codon usage tabulated from the international DNA sequence databases: status for the year 2000” *Nucl. Acids Res.* 28:292 (2000). Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge (Aptagen; Jacobus, Pa.), are also available. In some embodiments, one or more codons (e.g. 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more, or all codons) in a sequence encoding a CRISPR enzyme correspond to the most frequently used codon for a particular amino acid.

[0095] In some embodiments, a vector encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs. In some embodiments, the CRISPR enzyme comprises about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the amino-terminus, about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs at or near the carboxy-terminus, or a combination of these (e.g. one or more NLS at the amino-terminus and one or more NLS at the carboxy terminus). When more than one NLS is present, each may be selected independently of the others, such that a single NLS may be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. In a preferred embodiment of the invention, the CRISPR enzyme comprises at most 6 NLSs. In some embodiments, an NLS is considered near the N- or C-terminus when the nearest amino acid of the NLS is within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, or more amino acids along the polypeptide chain from the N- or C-ter-

minus. Typically, an NLS consists of one or more short sequences of positively charged lysines or arginines exposed on the protein surface, but other types of NLS are known. Non-limiting examples of NLSs include an NLS sequence derived from: the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV; the NLS from nucleoplasmin (e.g. the nucleoplasmin bipartite NLS with the sequence KRPAATKKAGQAKKKK); the c-myc NLS having the amino acid sequence PAAKRVKLD or RQRR-NELKRSP; the hRNP A1 M9 NLS having the sequence NQSSNFGPMKGGNFGGRSSGPYGGGGQY-FAKPRNQGGY; the sequence RMRIZFKNKGKDTAELRRRVEVSVELRKAKKDEQILKRRNV of the IBB domain from importin- α ; the sequences VSRKRPRP and PPKKARED of the myoma T protein; the sequence POP-KKKPL of human p53; the sequence SALIKKKKKMAP of mouse c-ab1 IV; the sequences DRLRR and PKQKKRK of the influenza virus NS1; the sequence RKLKKKIKKL of the Hepatitis virus delta antigen; the sequence REKKKFLKRR of the mouse Mx1 protein; the sequence KRKGDEVDGVDEVAKKKSKK of the human poly(ADP-ribose) polymerase; and the sequence RKCLQAGMNLKARKTKK of the steroid hormone receptors (human) glucocorticoid.

[0096] In general, the one or more NLSs are of sufficient strength to drive accumulation of the CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell. In general, strength of nuclear localization activity may derive from the number of NLSs in the CRISPR enzyme, the particular NLS(s) used, or a combination of these factors. Detection of accumulation in the nucleus may be performed by any suitable technique. For example, a detectable marker may be fused to the CRISPR enzyme, such that location within a cell may be visualized, such as in combination with a means for detecting the location of the nucleus (e.g. a stain specific for the nucleus such as DAPI). Examples of detectable markers include fluorescent proteins (such as Green fluorescent proteins, or GFP; RFP; CFP), and epitope tags (HA tag, flag tag, SNAP tag). Cell nuclei may also be isolated from cells, the contents of which may then be analyzed by any suitable process for detecting protein, such as immunohistochemistry, Western blot, or enzyme activity assay. Accumulation in the nucleus may also be determined indirectly, such as by an assay for the effect of CRISPR complex formation (e.g. assay for DNA cleavage or mutation at the target sequence, or assay for altered gene expression activity affected by CRISPR complex formation and/or CRISPR enzyme activity), as compared to a control not exposed to the CRISPR enzyme or complex, or exposed to a CRISPR enzyme lacking the one or more NLSs.

[0097] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of a CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (e.g. the Burrows Wheeler

Aligner), ClustalW, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about or more than about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. The ability of a guide sequence to direct sequence-specific binding of a CRISPR complex to a target sequence may be assessed by any suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay as described herein. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions. Other assays are possible, and will occur to those skilled in the art.

[0098] A guide sequence may be selected to target any target sequence. In some embodiments, the target sequence is a sequence within a genome of a cell. Exemplary target sequences include those that are unique in the target genome. For example, for the *S. pyogenes* Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXGG where NNNNNNNNNNNNNXGG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an *S. pyogenes* Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXGG where NNNNNNNNNNNNNXGG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. For the *S. thermophilus* CRISPR1 Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXXAGAAW where NNNNNNNNNNNNNXXAGAAW (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. A unique target sequence in a genome may include an *S. thermophilus* CRISPR1 Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXXAGAAW where NNNNNNNNNNNNNXXAGAAW (N is A, G, T, or C; X can be anything; and W is A or T) has a single occurrence in the genome. For the *S. pyogenes* Cas9, a unique target sequence in a genome may include a Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXXGGXG where NNNNNNNNNNNNNXXGGXG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. A unique target sequence in a genome may include an *S. pyogenes* Cas9 target site of the form MMMMMMMNNNNNNNNNNNNXXGGXG where NNNNNNNNNNNNNXXGGXG (N is A, G, T, or C; and X can be anything) has a single occurrence in the genome. In each of these sequences "M" may be A, G, T, or C, and need not be considered in identifying a sequence as unique.

[0099] In some embodiments, a guide sequence is selected to reduce the degree of secondary structure within the guide

sequence. Secondary structure may be determined by any suitable polynucleotide folding algorithm. Some programs are based on calculating the minimal Gibbs free energy. An example of one such algorithm is mFold, as described by Zuker and Stiegler (Nucleic Acids Res. 9 (1981), 133-148). Another example folding algorithm is the online webserver RNAfold, developed at Institute for Theoretical Chemistry at the University of Vienna, using the centroid structure prediction algorithm (see e.g. A. R. Gruber et al., 2008, *Cell* 106(1): 23-24; and PA Carr and GM Church, 2009, *Nature Biotechnology* 27(12): 1151-62). Further algorithms may be found in U.S. application Ser. No. TBA (Broad Reference BI-2012/084 44790.11.2022); incorporated herein by reference.

[0100] In general, a tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the corresponding tracr sequence; and (2) formation of a CRISPR complex at a target sequence, wherein the CRISPR complex comprises the tracr mate sequence hybridized to the tracr sequence. In general, degree of complementarity is with reference to the optimal alignment of the tracr mate sequence and tracr sequence, along the length of the shorter of the two sequences. Optimal alignment may be determined by any suitable alignment algorithm, and may further account for secondary structures, such as self-complementarity within either the tracr sequence or tracr mate sequence. In some embodiments, the degree of complementarity between the tracr sequence and tracr mate sequence along the length of the shorter of the two when optimally aligned is about or more than about 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97.5%, 99%, or higher. Example illustrations of optimal alignment between a tracr sequence and a tracr mate sequence are provided in FIGS. 12B and 13B. In some embodiments, the tracr sequence is about or more than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or more nucleotides in length. In some embodiments, the tracr sequence and tracr mate sequence are contained within a single transcript, such that hybridization between the two produces a transcript having a secondary structure, such as a hairpin. Preferred loop forming sequences for use in hairpin structures are four nucleotides in length, and most preferably have the sequence GAAA. However, longer or shorter loop sequences may be used, as may alternative sequences. The sequences preferably include a nucleotide triplet (for example, AAA), and an additional nucleotide (for example C or G). Examples of loop forming sequences include CAAA and AAAG. In an embodiment of the invention, the transcript or transcribed polynucleotide sequence has at least two or more hairpins. In preferred embodiments, the transcript has two, three, four or five hairpins. In a further embodiment of the invention, the transcript has at most five hairpins. In some embodiments, the single transcript further includes a transcription termination sequence; preferably this is a polyT sequence, for example six T nucleotides. An example illustration of such a hairpin structure is provided in the lower portion of FIG. 13B, where the portion of the sequence 5' of the final "N" and upstream of the loop corresponds to the tracr mate sequence, and the portion of the sequence 3' of the loop corresponds to the tracr sequence. Further non-limiting examples of single polynucleotides comprising a guide sequence, a tracr mate sequence, and a tracr sequence are as follows (listed 5' to 3'), where "N" represents a base of a guide sequence, the first block of lower case letters represent the

tracr mate sequence, and the second block of lower case letters represent the tracr sequence, and the final poly-T sequence represents the transcription terminator: (1) NNNNNNNNNgtttttgtactctcaagatt-taGAAAtaatacttcgacagaagctacaagataaggctt catgccgaatcaacacccgtgcattttatggcagggtgttttcgtatttaaTTTTTT; (2) NNNNNNNNNNNNNNNNNNNNNNNgtttttg-tactctcaGAAAtgcagaagctacaagataaggcttcagccgaatcaacacccgtgcattttatggcagggtgttttcgtatttaaTTTTTT; (3) NNNNNNNNNNNNNNNNNNNNNNNgtttttg-tactctcaGAAAtgcagaagctacaagataaggcttcagccgaatcaacacccgtgcattttatggcagggtgtTTTTTT; (4) NNNNNNNNNNNNNNNNNNNNNNNgtttta-gagctaGAAAtgcaagttaaataaggctagtcggttatcaactgaaaaagtggcaccgagtcgggtgcTTTTTT; (5) NNNNNNNNNNNNNNNNNNNNNNNgtttta-gagctaGAAATAGcaagttaaataaggctagtcggttatcaactgaaaagtgtTTTTTT; and (6) NNNNNNNNNNNNNNNNNNNNNNNgtttta-gagctagAAATAGcaagttaaataaggctagtcggttatcaTTTTTT. In some embodiments, sequences (1) to (3) are used in combination with Cas9 from *S. thermophilus* CRISPR1. In some embodiments, sequences (4) to (6) are used in combination with Cas9 from *S. pyogenes*. In some embodiments, the tracr sequence is a separate transcript from a transcript comprising the tracr mate sequence (such as illustrated in the top portion of FIG. 13B).

[0101] In some embodiments, a recombination template is also provided. A recombination template may be a component of another vector as described herein, contained in a separate vector, or provided as a separate polynucleotide. In some embodiments, a recombination template is designed to serve as a template in homologous recombination, such as within or near a target sequence nicked or cleaved by a CRISPR enzyme as a part of a CRISPR complex. A template polynucleotide may be of any suitable length, such as about or more than about 10, 15, 20, 25, 50, 75, 100, 150, 200, 500, 1000, or more nucleotides in length. In some embodiments, the template polynucleotide is complementary to a portion of a polynucleotide comprising the target sequence. When optimally aligned, a template polynucleotide might overlap with one or more nucleotides of a target sequences (e.g. about or more than about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100 or more nucleotides). In some embodiments, when a template sequence and a polynucleotide comprising a target sequence are optimally aligned, the nearest nucleotide of the template polynucleotide is within about 1, 5, 10, 15, 20, 25, 50, 75, 100, 200, 300, 400, 500, 1000, 5000, 10000, or more nucleotides from the target sequence.

[0102] In some embodiments, the CRISPR enzyme is part of a fusion protein comprising one or more heterologous protein domains (e.g. about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more domains in addition to the CRISPR enzyme). A CRISPR enzyme fusion protein may comprise any additional protein sequence, and optionally a linker sequence between any two domains. Examples of protein domains that may be fused to a CRISPR enzyme include, without limitation, epitope tags, reporter gene sequences, and protein domains having one or more of the following activities: methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity and nucleic acid binding activity. Non-limiting examples of epitope tags include histidine (His) tags,

V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta-galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). A CRISPR enzyme may be fused to a gene sequence encoding a protein or a fragment of a protein that bind DNA molecules or bind other cellular molecules, including but not limited to maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GALA DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. Additional domains that may form part of a fusion protein comprising a CRISPR enzyme are described in US20110059502, incorporated herein by reference. In some embodiments, a tagged CRISPR enzyme is used to identify the location of a target sequence.

[0103] In some aspects, the invention provides methods comprising delivering one or more polynucleotides, such as or one or more vectors as described herein, one or more transcripts thereof, and/or one or more proteins transcribed therefrom, to a host cell. In some aspects, the invention further provides cells produced by such methods, and organisms (such as animals, plants, or fungi) comprising or produced from such cells. In some embodiments, a CRISPR enzyme in combination with (and optionally complexed with) a guide sequence is delivered to a cell. Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids in mammalian cells or target tissues. Such methods can be used to administer nucleic acids encoding components of a CRISPR system to cells in culture, or in a host organism. Non-viral vector delivery systems include DNA plasmids, RNA (e.g. a transcript of a vector described herein), naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell. For a review of gene therapy procedures, see Anderson, *Science* 256:808-813 (1992); Nabel & Felgner, *TIBTECH* 11:211-217 (1993); Mitani & Caskey, *TIBTECH* 11:162-166 (1993); Dillon, *TIBTECH* 11:167-175 (1993); Miller, *Nature* 357:455-460 (1992); Van Brunt, *Biotechnology* 6(10):1149-1154 (1988); Vigne, *Restorative Neurology and Neuroscience* 8:35-36 (1995); Kremer & Perricaudet, *British Medical Bulletin* 51(1):31-44 (1995); Haddada et al., in *Current Topics in Microbiology and Immunology* Doerfler and Böhm (eds) (1995); and Yu et al., *Gene Therapy* 1:13-26 (1994).

[0104] Methods of non-viral delivery of nucleic acids include lipofection, nucleofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Feigner, WO 91/17424; WO 91/16024. Delivery can be to cells (e.g. in vitro or ex vivo administration) or target tissues (e.g. in vivo administration).

[0105] The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid com-

plexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[0106] The use of RNA or DNA viral based systems for the delivery of nucleic acids takes advantage of highly evolved processes for targeting a virus to specific cells in the body and trafficking the viral payload to the nucleus. Viral vectors can be administered directly to patients (in vivo) or they can be used to treat cells in vitro, and the modified cells may optionally be administered to patients (ex vivo). Conventional viral based systems could include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors for gene transfer. Integration in the host genome is possible with the retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in long term expression of the inserted transgene. Additionally, high transduction efficiencies have been observed in many different cell types and target tissues.

[0107] The tropism of a retrovirus can be altered by incorporating foreign envelope proteins, expanding the potential target population of target cells. Lentiviral vectors are retroviral vectors that are able to transduce or infect non-dividing cells and typically produce high viral titers. Selection of a retroviral gene transfer system would therefore depend on the target tissue. Retroviral vectors are comprised of cis-acting long terminal repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum cis-acting LTRs are sufficient for replication and packaging of the vectors, which are then used to integrate the therapeutic gene into the target cell to provide permanent transgene expression. Widely used retroviral vectors include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human immuno deficiency virus (HIV), and combinations thereof (see, e.g., Buchscher et al., J. Virol. 66:2731-2739 (1992); Johann et al., J. Virol. 66:1635-1640 (1992); Sommerfelt et al., Virol. 176:58-59 (1990); Wilson et al., J. Virol. 63:2374-2378 (1989); Miller et al., J. Virol. 65:2220-2224 (1991); PCT/US94/05700).

[0108] In applications where transient expression is preferred, adenoviral based systems may be used. Adenoviral based vectors are capable of very high transduction efficiency in many cell types and do not require cell division. With such vectors, high titer and levels of expression have been obtained. This vector can be produced in large quantities in a relatively simple system. Adeno-associated virus ("AAV") vectors may also be used to transduce cells with target nucleic acids, e.g., in the in vitro production of nucleic acids and peptides, and for in vivo and ex vivo gene therapy procedures (see, e.g., West et al., Virology 160:38-47 (1987); U.S. Pat. No. 4,797,368; WO 93/24641; Kotin, Human Gene Therapy 5:793-801 (1994); Muzyczka, J. Clin. Invest. 94:1351 (1994). Construction of recombinant AAV vectors are described in a number of publications, including U.S. Pat. No. 5,173,414; Tratschin et al., Mol. Cell. Biol. 5:3251-3260 (1985); Tratschin, et al., Mol. Cell. Biol. 4:2072-2081 (1984); Hermonat & Muzyczka, PNAS 81:6466-6470 (1984); and Samulski et al., J. Virol. 63:03822-3828 (1989).

[0109] Packaging cells are typically used to form virus particles that are capable of infecting a host cell. Such cells

include 293 cells, which package adenovirus, and ψ 2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producing a cell line that packages a nucleic acid vector into a viral particle. The vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the polynucleotide(s) to be expressed. The missing viral functions are typically supplied in trans by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely rep and cap, but lacking ITR sequences. The cell line may also also be infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV. Additional methods for the delivery of nucleic acids to cells are known to those skilled in the art. See, for example, US20030087817, incorporated herein by reference.

[0110] In some embodiments, a host cell is transiently or non-transiently transfected with one or more vectors described herein. In some embodiments, a cell is transfected as it naturally occurs in a subject. In some embodiments, a cell that is transfected is taken from a subject. In some embodiments, the cell is derived from cells taken from a subject, such as a cell line. A wide variety of cell lines for tissue culture are known in the art. Examples of cell lines include, but are not limited to, C8161, CCRF-CEM, MOLT, mIMCD-3, NHDF, HeLa-S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEK293, HEK293T, MiaPaCell, Panc1, PC-3, TF1, CTLL-2, C1R, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu1, SW480, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, T1B55, Jurkat, J45.01, LMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, 132-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, A2780ADR, A2780cis, A172, A20, A253, A431, A-549, ALC, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293, BxPC3, C3H-10T1/2, C6/36, Cal-27, CHO, CHO-7, CHO-IR, CHO-K1, CHO-K2, CHO-T, CHO Dhfr^{-/-}, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV-434, CML T1, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, HEK-293, HeLa, Hepa1c1c7, HL-60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KYO1, LNCap, Ma-Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK II, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NALM-1, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-SF, RMA/RMAS, Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-1, YAR, and transgenic varieties thereof. Cell lines are available from a variety of sources known to those with skill in the art (see, e.g., the American Type Culture Collection (ATCC) (Manas-

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

[0111] In some embodiments, one or more vectors described herein are used to produce a non-human transgenic animal or transgenic plant. In some embodiments, the transgenic animal is a mammal, such as a mouse, rat, or rabbit. In certain embodiments, the organism or subject is a plant. In certain embodiments, the organism or subject or plant is algae. Methods for producing transgenic plants and animals are known in the art, and generally begin with a method of cell transfection, such as described herein. Transgenic animals are also provided, as are transgenic plants, especially crops and algae. The transgenic animal or plant may be useful in applications outside of providing a disease model. These may include food or feed production through expression of, for instance, higher protein, carbohydrate, nutrient or vitamins levels than would normally be seen in the wildtype. In this regard, transgenic plants, especially pulses and tubers, and animals, especially mammals such as livestock (cows, sheep, goats and pigs), but also poultry and edible insects, are preferred.

[0112] Transgenic algae or other plants such as rape may be particularly useful in the production of vegetable oils or bio-fuels such as alcohols (especially methanol and ethanol), for instance. These may be engineered to express or overexpress high levels of oil or alcohols for use in the oil or biofuel industries.

[0113] In one aspect, the invention provides for methods of modifying a target polynucleotide in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the target polynucleotide to effect cleavage of said target polynucleotide thereby modifying the target polynucleotide, wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said target polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence.

[0114] In one aspect, the invention provides a method of modifying expression of a polynucleotide in a eukaryotic cell. In some embodiments, the method comprises allowing a CRISPR complex to bind to the polynucleotide such that said binding results in increased or decreased expression of said polynucleotide; wherein the CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within said polynucleotide, wherein said guide sequence is linked to a tracr mate sequence which in turn hybridizes to a tracr sequence.

[0115] With recent advances in crop genomics, the ability to use CRISPR-Cas systems to perform efficient and cost effective gene editing and manipulation will allow the rapid selection and comparison of single and multiplexed genetic manipulations to transform such genomes for

improved production and enhanced traits. In this regard reference is made to US patents and publications: U.S. Pat. No. 6,603,061—*Agrobacterium*-Mediated Plant Transformation Method; U.S. Pat. No. 7,868,149—Plant Genome Sequences and Uses Thereof and US 2009/0100536—Transgenic Plants with Enhanced Agronomic Traits, all the contents and disclosure of each of which are herein incorporated by reference in their entirety. In the practice of the invention, the contents and disclosure of Morrell et al “Crop genomics: advances and applications” Nat Rev Genet. 2011 Dec. 29; 13(2):85-96 are also herein incorporated by reference in their entirety. In an advantageous embodiment of the invention, the CRISPR/Cas9 system is used to engineer microalgae (Example 14). Accordingly, reference herein to animal cells may also apply, mutatis mutandis, to plant cells unless otherwise apparent.

[0116] In one aspect, the invention provides for methods of modifying a target polynucleotide in a eukaryotic cell, which may be in vivo, ex vivo or in vitro. In some embodiments, the method comprises sampling a cell or population of cells from a human or non-human animal or plant (including microalgae), and modifying the cell or cells. Culturing may occur at any stage ex vivo. The cell or cells may even be re-introduced into the non-human animal or plant (including microalgae).

[0117] In one aspect, the invention provides kits containing any one or more of the elements disclosed in the above methods and compositions. In some embodiments, the kit comprises a vector system and instructions for using the kit. In some embodiments, the vector system comprises (a) a first regulatory element operably linked to a tracr mate sequence and one or more insertion sites for inserting a guide sequence upstream of the tracr mate sequence, wherein when expressed, the guide sequence directs sequence-specific binding of a CRISPR complex to a target sequence in a eukaryotic cell, wherein the CRISPR complex comprises a CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence; and/or (b) a second regulatory element operably linked to an enzyme-coding sequence encoding said CRISPR enzyme comprising a nuclear localization sequence. Elements may provide individually or in combinations, and may be provided in any suitable container, such as a vial, a bottle, or a tube. In some embodiments, the kit includes instructions in one or more languages, for example in more than one language.

[0118] In some embodiments, a kit comprises one or more reagents for use in a process utilizing one or more of the elements described herein. Reagents may be provided in any suitable container. For example, a kit may provide one or more reaction or storage buffers. Reagents may be provided in a form that is usable in a particular assay, or in a form that requires addition of one or more other components before use (e.g. in concentrate or lyophilized form). A buffer can be any buffer, including but not limited to a sodium carbonate buffer, a sodium bicarbonate buffer, a borate buffer, a Tris buffer, a MOPS buffer, a HEPES buffer, and combinations thereof. In some embodiments, the buffer is alkaline. In some embodiments, the buffer has a pH from about 7 to about 10. In some embodiments, the kit comprises one or more oligonucleotides corresponding to a guide sequence for insertion into a vector so as to operably link the guide sequence and a regulatory element. In some embodiments, the kit comprises a homologous recombination template polynucleotide.

[0119] In one aspect, the invention provides methods for using one or more elements of a CRISPR system. The

CRISPR complex of the invention provides an effective means for modifying a target polynucleotide. The CRISPR complex of the invention has a wide variety of utility including modifying (e.g., deleting, inserting, translocating, inactivating, activating) a target polynucleotide in a multiplicity of cell types. As such the CRISPR complex of the invention has a broad spectrum of applications in, e.g., gene therapy, drug screening, disease diagnosis, and prognosis. An exemplary CRISPR complex comprises a CRISPR enzyme complexed with a guide sequence hybridized to a target sequence within the target polynucleotide. The guide sequence is linked to a tracr mate sequence, which in turn hybridizes to a tracr sequence.

[0120] The target polynucleotide of a CRISPR complex can be any polynucleotide endogenous or exogenous to the eukaryotic cell. For example, the target polynucleotide can be a polynucleotide residing in the nucleus of the eukaryotic cell. The target polynucleotide can be a sequence coding a gene product (e.g., a protein) or a non-coding sequence (e.g., a regulatory polynucleotide or a junk DNA). Without wishing to be bound by theory, it is believed that the target sequence should be associated with a PAM (protospacer adjacent motif); that is, a short sequence recognised by the CRISPR complex. The precise sequence and length requirements for the PAM differ depending on the CRISPR enzyme used, but PAMs are typically 2-5 base pair sequences adjacent the protospacer (that is, the target sequence) Examples of PAM sequences are given in the examples section below, and the skilled person will be able to identify further PAM sequences for use with a given CRISPR enzyme.

[0121] The target polynucleotide of a CRISPR complex may include a number of disease-associated genes and polynucleotides as well as signaling biochemical pathway-associated genes and polynucleotides as listed in US provisional patent applications 61/736,527 and 61/748,427 having Broad reference BI-2011/008/WSGR Docket No. 44063-701.101 and BI-2011/008/WSGR Docket No. 44063-701.102 respectively, both entitled SYSTEMS METHODS AND COMPOSITIONS FOR SEQUENCE MANIPULATION filed on Dec. 12, 2012 and Jan. 2, 2013, respectively, the contents of all of which are herein incorporated by reference in their entirety.

[0122] Examples of target polynucleotides include a sequence associated with a signaling biochemical pathway, e.g., a signaling biochemical pathway-associated gene or polynucleotide. Examples of target polynucleotides include a disease associated gene or polynucleotide. A “disease-associated” gene or polynucleotide refers to any gene or polynucleotide which is yielding transcription or translation products at an abnormal level or in an abnormal form in cells derived from a disease-affected tissues compared with tissues or cells of a non disease control. It may be a gene that becomes expressed at an abnormally high level; it may be a gene that becomes expressed at an abnormally low level, where the altered expression correlates with the occurrence and/or progression of the disease. A disease-associated gene also refers to a gene possessing mutation(s) or genetic variation that is directly responsible or is in linkage disequilibrium with a gene(s) that is responsible for the etiology of a disease. The transcribed or translated products may be known or unknown, and may be at a normal or abnormal level.

[0123] Examples of disease-associated genes and polynucleotides are available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore,

Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), available on the World Wide Web.

[0124] Examples of disease-associated genes and polynucleotides are listed in Tables A and B. Disease specific information is available from McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, Md.) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, Md.), available on the World Wide Web. Examples of signaling biochemical pathway-associated genes and polynucleotides are listed in Table C.

[0125] Mutations in these genes and pathways can result in production of improper proteins or proteins in improper amounts which affect function. Further examples of genes, diseases and proteins are hereby incorporated by reference from US Provisional applications 61/736,527 and 61/748,427. Such genes, proteins and pathways may be the target polynucleotide of a CRISPR complex.

TABLE A

DISEASE/DISORDER	GENE(S)
Neoplasia	PTEN; ATM; ATR; EGFR; ERBB2; ERBB3; ERBB4; Notch1; Notch2; Notch3; Notch4; AKT; AKT2; .AKT3; HIF; HIF1a; HIF3a; Met; HRG; Bcl2; PPAR alpha; PPAR gamma; WT1 (Wilms Tumor); FGF Receptor Family members (5 members: 1, 2, 3, 4, 5); CDKN2a; APC; RB (retinoblastoma); MEN1; VHL; BRCA1; BRCA2; AR (Androgen Receptor); TSG101; IGF; IGF Receptor; Igf1 (4 variants); Igf2 (3 variants); Igf 1 Receptor; Igf 2 Receptor; Bax; Bcl2; caspases family (9 members: 1, 2, 3, 4, 6, 7, 8, 9, 12); Kras; Apc Abcr; Ccl2.; Cc2.; cp (ceruloplasmin); Timp3; cathepsinD; Vldlr; Ccr2
Age-related Macular Degeneration	Neuregulin 1 (Nrg 1); Erb4 (receptor for Neuregulin); Complexin1 (Cplx1); Tph1
Schizophrenia	Tryptophan hydroxylase; Tph2
Disorders	Tryptophan hydroxylase 2; Neurexin 1; GSK3; GSK3a; 5-HTT (slc6a4); COMT; DRD (Drd1a); SLC6A3 DTNBP1; Dao (Dao1)
Trinucleotide Repeat Disorders	HTT (Huntington's Dx); SBMA/SMAX1/AR (Kennedy's Dx); FXN/X25 (Friedrich's Ataxia); ATX3 (Machado-Joseph's Dx); ATXN1, and ATXN2 (spinocerebellar ataxias); I/MPK (myotonic dystrophy); Atrophin-1 and Atn1 (DRPLA Dx); CBP (Creb-BP-global instability); VLDLR (Alzheimer's); Atxn7; Atxn10
Fragile X Syndrome	FMR2; FXR1; FXR2; mGLUR5
Secretase Related Disorders	APH-1 (alpha and beta); Presenilin (Psen1); nicastrin (Ncstn); PEN-2

TABLE A-continued

DISEASE/DISORDER	GENE(S)
Others	Nos1; Parp1; Nat 1; Nat2.
Prion-related disorders	Prp
ALS	SOD1; ALS2; STEX; FUS; TARDBP; VEGF (VEGF-a; VEGF-b; VEGF-c)
Drug addiction	Prkce (alcohol); Drd2; Drd4; ABAT (alcohol); GRIA2; Grm5; Grin1; Htr1b; Grin2a; Drd3; Pdyn; Grial (alcohol)
Autism	Mecp2; BZRAP1; MDGA2; Sema5A; Neurexin 1; Fragile X (FMR2 (AFF2); FXR1; FXR2; Mglur5)
Alzheimer's Disease	E1; CHIP; UGH; UBB; Tau; LRP; PICALM; Clusterin; PS1; SORL1; CR1; V1dlr; Ubal; Uba3; CHIP28 (Aqp1, Aquaporin 1); Uchl1; Uchl3; APP
Inflammation	IL-10; IL-1 (IL-1a; IL-1b); IL-13; IL-17 (IL-17a (CTLA8); IL- 17b; IL-17c; IL-17d; IL-17f); IL-23; Cx3cr1; ptpn22; TNFa; NOD2/CARD15 for IBD; IL-6; IL-12 (IL-12a; IL-12b); CTLA4; Cx3cl1
Parkinson's Disease	x-Synuclein; DJ-1; LRRK2; Parkin; PINK1

TABLE B

Blood and coagulation diseases and disorders	Anemia (CDAN1, CDAI, RPS19, DBA, PKLR, PKI, NT5C3, UMPHI, PSN1, RHAG, RH50A., NRAMP2, SBTP, ALAS2, ANH1, ASB, ABCB7, ABC7, ASAT); Bare lymphocyte syndrome (TAPBP, TPSN, TAP2, ABCB3, PSF2, RING11, MHC2TA, C2TA, RFX5, RFXAP, RFX5), Bleeding disorders (TBXA2R, P2RX1, P2X1); Factor H and factor H-like 1 (HF1, CFH, HUS); Factor V and factor VIII (MCFD2); Factor VII deficiency (F7); Factor X deficiency (F10); Factor XI deficiency (F11); Factor XII deficiency (F12, HAF); Factor XIIIa deficiency (F13A1, F13A); Factor XIIIb deficiency (F13B); Fanconi anemia (FANCA, FACA, FA1, FA, FAA, FAAP95, FAAP90, FULJ34064, FANCB, FANCC, FACC, BRCA2, FANCD1, FANCD2, FANCD, FACD, FAD, FANCE, FACE, FANCF, XRCC9, FANCG, BRIP1, BACH1, FANCI, PHF9, FANCL, FANCM, KIAA1596); Hemophagocytic lymphohistiocytosis disorders (PRF1, HPLH2, UNC13D, MUNC13-4, HPLH3, HLH3, FHL3); Hemophilia A (F8, F8C, HEMA); Hemophilia B (F9, HEMB), Hemorrhagic disorders (PI, ATT, F5); Leukocyte deficiencies and disorders (ITGB2, CD18, LCAMB, LAD, EIF2B1, EIF2B2, EIF2B3, EIF2B5, LVWM, CACH, CLE, EIF2B4); Sickle cell anemia (HBB); Thalassemia (HBA2, HBB, HBD, LCRB, HBA1).
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TABLE B-continued

Cell dysregulation and oncology diseases and disorders	B-cell non-Hodgkin lymphoma (BCL7A BCL7); Leukemia (TAL1, TCL5, SCL, TAL2, FLT3, NBS1, NBS, ZNFN1A1, IK1, LYF1, HOXD4, HOX4B; BCR, CML, PHL, ALL, ARNT, KRAS2, RASK2, GMPS, AF10, ARHGEF12, LARG, KIAA0382, CALM, CLTH, CEBPA, CEBP, CHIC2, BTL, FLT3, KIT, PBT, LPP, NPM1, NUP214, D9S46E, CAN, CAIN, RUNX1, CBFA2, AML1, WHSC1L1, NSD3, FLT3, AF1Q, NPM1, NUMA1, ZNF145, PLZF, PML, MYL, STAT5B, AF10, CALM, CLTH, ARL11, ARLTS1, P2RX7, P2X7, BCR, CML, PHL, ALL, GRAF, NF1, VRNF, WSS, NFNS, PTPN11, PTP2C, SHP2, NS1, BCL2, CCND1, PRAD1, BCL1, TCRA, GATA1, GF1, ERYF1, NFE1, ABL1, NQO1, DIA4, NMOR1, NUP214, D9S46E, CAN, CAIN),
Inflammation and immune related diseases and disorders	AIDS (KIR3DL1, NKAT3, NKB1, AMB11, KIR3DS1, IFNG, CXCL12, SDF1); Autoimmune lymphoproliferative syndrome (TNFRSF6, APT1, FAS, CD95, ALPS1A); Combined immunodeficiency, (IL2RG, SCIDX1, SCIDX, IMD4); HIV-1 (CCL5, SCYA5, D17S136E, TCP228), HIV susceptibility or infection (IL10, CSIF, CMKBR2, CCR2, CMKBR5, CCCR5 (CCR5)); Immunodeficiencies (CD3E, CD3G, AICDA, AID, HIGM2, TNFRSF5, CD40, UNG, DGU, HIGM4, TNFSF5, CD40LG, HIGM1, IGM, FOXP3, IPEX, AIID, XPID, PIDX, TNFRSF14B, TAC1; Inflammation (IL-10, IL-1 (IL-1a, IL-1b), IL-13, IL-17 (IL-17a (CTLA8), IL-17b, IL-17c, IL-17d, IL-17f), IL-23, Cx3cr1, ptpn22, TNFa, NOD2/CARD15 for IBD, IL-6, IL-12 (IL-12a, IL-12b), CTLA4, Cx3l1); Severe combined immunodeficiencies (SCIDs)(JAK3, JAKL, DCLRE1C, ARTEMIS, SCIDA, RAG1, RAG2, ADA, PTPRC, CD45, LCA, IL7R, CD3D, T3D, IL2RG, SCIDX1, SCIDX, IMD4).
Metabolic, liver, kidney and protein diseases and disorders	Amyloid neuropathy (TTR, PALB); Amyloidosis (APOA1, APP, AAA, CVAP, AD1, GSN, FGA, LYZ, TTR, PALB); Cirrhosis (KRT18, KRT8, CIRHIA, NAIC, TEX292, KIAA1988); Cystic fibrosis (CFTR, ABCC7, CF, MRP7); Glycogen storage diseases (SLC2A2, GLUT2, G6PC, G6PT, G6PT1, GAA, LAMP2, LAMPB, AGL, GDE, GBE1, GYS2, PYGL, PFKM); Hepatic adenoma, 142330 (TCF1, HNF1A, MODY3), Hepatic failure, early onset, and neurologic disorder (SCOD1, SCO1), Hepatic lipase deficiency (LIPC), Hepatoblastoma, cancer and carcinomas (CTNNB1, PDGFRL, PDGRL, PRLTS, AXIN1, AXIN, CTNNB1, TP53, P53, LFS1, IGF2R, MPRI, MET, CASP8, MCH5; Medullary cystic kidney disease (UMOD, HNFJ, FJHN, MCKD2, ADMCKD2); Phenylketonuria (PAH, PKU1, QDPR, DHPR, PTS); Polycystic kidney and hepatic disease

TABLE B-continued

Muscular/ Skeletal diseases and disorders	(FCYT, PKHD1, ARPKD, PKD1, PKD2, PKD4, PKDTS, PRKCSH, G19P1, PCLD, SEC63); Becker muscular dystrophy (DMD, BMD, MYF6), Duchenne Muscular Dystrophy (DMD, BMD); Emery-Dreifuss muscular dystrophy (LMNA, LMN1, EMD2, FPLD, CMD1A, HGPS, LGMD1B, LMNA, LMN1, EMD2, FPLD, CMD1A); Facioscapulohumeral muscular dystrophy (FSHMD1A, FSHD1A); Muscular dystrophy (FKRP, MDC1C, LGMD2I, LAMA2, LAMM, LARGE, KIAA0609, MDC1D, FCMO, TTID, MYOT, CAPN3, CANP3, DYSF, LGMD2B, SGCG, LGMD2C, DMDA1, SCG3, SOCA, ADL, DAG2, LGM2D, DMDA2, SGCB, LGMD2E, SGCD, SGD, LGMD2F, CMD1L TCAP, LGMD2G, CMD1N, TRIM32, HT2A, LGMD2H, FKRP, MDC1C, LGMD2I, TTN, CMD1G, TMD, LGMD2J, POMT1, CAV3, LGMD1C, SEPN1, SELN, RSMD1, PLEC1, PLTN, EBS1); Osteopetrosis (LRP5, BMND1, LRP7, LR3, OPPG, VBCH2, CLCN7, CLC7, OPTA2, OSTM1, GL, TCIRG1, TIRC7, OC116, OPTB1); Muscular atrophy (VAPB, VAPC, ALS8, SMN1, SMA1, SMA2, SMA3, SMA4, BSCL2, SPG17, GARS, SMAD1, CMT2D, HEXB, IGHMBP2, SMUBP2, CATF1, SMARD1);
	Neurological and neuronal diseases and disorders
	ALS (SOD1, ALS2, STEX, FUS, TARDBP, VEGF (VEGF-a, VEGF-b, VEGF-c); Alzheimer disease (APP, AAA, CVAP, AD1, APOE, AD2, PSEN2, AD4, STM2, APBB2, FE65L1, NOS3, PLA2, URK, ACE, DCP1, ACE1, MPO, PAC1P1 PAXIP1L, PTIP, A2M, BLMH, BMH, PSEN1, AD3); Autism (Mecp2, BZRAP1, MDGA2, Sema5A, Neurexin 1, GLO1, MECP2, RTT, PPMX, MRX16, MRX79, NLGN3, NLGN4, KIAA1260, AUTSX2); Fragile X Syndrome (FMR2, FXR1, FXR2, mGLUR5); Huntington's disease and disease like disorders (HD, IT15, PRNP, PRIP, JPH3, JP3, HDL2, TBP, SCA17); Parkinson disease (NR4A2, NURR1, NOT, TINUR, SNCAIP, TBP, SCA17, SNCA, NACP; PARK1, PARK4, DJ1, PARK7, LRRK2, PARK8, PINK1, PARK6, UCHL1, PARK5, SNCA, NACP, PARK1, PARK4, PRKN, PARK2, PDJ, DBH, NDUF-V2); Rett syndrome (MECP2, RTT, PPMX, MRX16, MRX79, CDKL5, , STK9, MECP2, RTT, PPMX, MRX16, MRX79, x-Synuclein, DJ-1); Schizophrenia (Neuregulin1 (Nrg1) Erb4 (receptor for Neuregulin), Complexin1 (Cplx1), Tph1 Tryptophan hydroxylase, Tph2, Tryptophan hydroxylase 2, Neurexin 1, GSK3, GSK3a, GSK3b, 5-HTT (S1c6a4), COW, DRD (Drd1a), SLC6A3, DAOA, DTNBP1, Dao (Dao1)); Secretase Related Disorders (APH-1 (alpha and beta), Presenitin (Pseni), nicastrin, (Ncstn), PEN-2, Nos1, Parp1, Nat1, Nat2); Trinucleotide Repeat Disorders (HTT (Huntington's Dx), SBMA/SMAX1/AR (Kennedy's Dx), FXN/X25 (Friedrich's Ataxia), ATX3 (Machado- Joseph's Dx), ATXN1 and ATXN2

TABLE B-continued

Ocular diseases and disorders	(spinocerebellar ataxias), DMPK (myotonic dystrophy), Atrophin-1 and Atn1 (DRPLA Dx), CBP (Creb-BP-global instability), VLDLR (Alzheimer's), Atxn7, Atxn10). Age-related macular degeneration (Abcr, Ccl2, Cc2, cp (ceruloplasmin), Timp3, cathepsinD, Vldlr, Ccr2); Cataract (CRYAA, CRYA1, CRYBB2, CRYB2, PITX3, BFSP2, CP49, CP47, CRYAA, CRYA1, PAX6, AN2, MGDA, CRYBA1, CRYB1, CRYGC, CRYG3, CCL, LIM2, MP19, CRYGD, CRYG4, BFSP2, CP49, CP47, HSF4, CTM, HSF4, CTM, MIP, AQP0, CRYAB, CRYA2, CTTP2, CRYBB1, CRYGD, CRYG4, CRYBB2, CRYB2, CRYGC, CRYG3, CCL, CRYAA, CRYA1, GJA8, CX50, CAE1, CJA3, CX46, CZP3, CAE3, CCM1, CAM, KRIT1); Corneal clouding and dystrophy (APOA1, TGFB1, CSD2, CDGGI, CSD, BIGH3, CDG2, TACSTD2, TROP2, MIS1, VSX1, RINX, PPCD, PPD, KTCN, COL8A2, FECD, PPCD2, PIP5K3, CFD); Cornea plana congenital (KERA, CNA2); Glaucoma (MYOC, TIGR, GLCIA, JOAG, GPOA, OPTN, GLC1E, FIP2, HYPL, NRP, CYP1B1, GLC3A, OPA1 NTG, NPG, CYP1B1, GLC3A); Leber congenital amaurosis (CRB1, RP12, CRX, CORD2, CRD, RPGRIP1, LCA6, CORD9, RPE65, RP20, AIPL1, LCA4, GUCY2D, GUC2D, LCA1, CORD6, RDH12, LCA3); Macular dystrophy (ELOVL4, ADMD, STGD2, STGD3, RDS, RP7, PRPH2, PRPH, AVMD, AOFMD, VMD2).

TABLE C

CELLULAR FUNCTION	GENES
PI3K/AKT Signaling	PRKCE.; ITGAM; ITGA5; IRAK1; PRKAA2; EIF2AK2; PTEN; EIF4E; PRKCZ; GRK6; MAPK1; TSC1; PLK1; AKT2; IKBKB; PIK3CA; CDK8; CDKN1B; NFKB2; BCL2; PIK3CB; PPP2R1A; MAPK8; BCL2L1; MAPK3; TSC2; ITGA1; KRAS; EIF4EBP1; RELA; PRKCD; NOS3; PRKAA1; MAPK9; CDK2; PPP2CA; PIM1; ITGB7; YWHAZ; ILK; TP53; RAF1; IKBKG; RELB; DYRK1A; CDKN1A; ITGB1; MAP2K2; JAM; AKT1; JAK2; PIK3R1; CHUK; PDPK1; PPP2R5C; CTNNB1; MAP2K1; NFKB1; PAK3; ITGB3; CCND1; GSK3A; FRAP1; SFN; ITGA2; TTK.; CSNK1A1 ; BRAF; GSK3B; AKT3; FOXO1; SGK.; HSP90AA1; RPS6KB1 PRKCE; ITGAM; ITGA5; HSPB1; IRAK1; PRKAA2; EIF2AK2; RAC1; RAP1A; TLN1; EIF4E; ELK1; GRK6; MAPK1; RAC2; PLK1;
ERK/MAPK Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
	FYN; DYRK1A; ITGB1; MAP2K2; PAK4; AKT1; JAK2; STAT3; ADAM10; MAP2K1; PAK3; ITGB3; CDC42; VEGFA; ITGA2; EPHA8; TTK; CSNK1A1; CRKL; BRAF; PTPN13; ATF4; AKT3; SGK
Actin	ACTN4; PRKCE; ITGAM; ROCK1;
Cytoskeleton	ITGA5; IRAK1;
Signaling	PRKAA2; EIF2AK2; RAC1; INS; ARHGEF7; GRK6; ROCK2; MAPK1; RAC2; PLK1; AKT2; PIK3CA; CDK8; PTK2; CFL1 ; PIK3CB; MYH9; DIAPH1; PIK3C3; MAPK8; F2R; MAPK3; SLC9A1; ITGA1; KRAS; RHOA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; ITGB7; PPP1CC; PXN; VIL2; RAF1; GSN; DYRK1A; ITGB1; MAP2K2; PAK4; P1P5K1A; PIK3R1; MAP2K1; PAK3; ITGB3; CDC42; APC; ITGA2; TTK; CSNK1A1; CRKL; BRAF; VAV3; SGK
Huntington's Disease	PRKCE; IGF1; EP300; RCOR1; PRKCE; HDAC4; TGM2; MAPK1; CAPNS1; AKT2; EGFR; NCOR2; SP1; CAPN2; PIK3CA; HDAC5; CREB1; PRKCI; HSPA5; REST;
Signaling	GNAQ; PIK3CB; PIK3C3; MAPK8; IGF1R; PRKD1; GNB2L1; BCL2L1; CAPN1; MAPK3; CASP8; HDAC2; HDAC7A; PRKCD; HDAC11; MAPK9; HDAC9; PIK3C2A; HDAC3; TP53; CASP9; CREBBP; AKT1; PIK3R1; PDPK1; CASP1; APAF1; FRAP1; CASP2; JUN; BAX; ATF4; AKT3; PRKCA; CLTC; SGK; HDAC6; CASP3
Apoptosis	PRKCE; ROCK1; BID; IRAK1; PRKAA2; EIF2AK2; BAK1; BIRC4; GRK6; MAPK1; CAPNS1; PLK1; AKT2; .IKBKB; CAPN2; CDK8; FAS; NFKB2; BCL2; MAP3K14; MAPK8; BCL2LI; CAPN1; MAPK3; CASPS; KRAS; RELA; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; TP53; TNF; RAFI; IKBKG; RELB; CASP9; DYRK1A; MAP2K2; CHUK; APAF1; MAP2K1; NFKBI; PAK3; LMNA; CASP2; BIRC2; TTK; CSNK1A1; BRAF; BAX; PRKCA; SGK; CASP3; BIRC3; PARP1
Apoptosis Signaling	RAC1; PTEN; LYN; ELK1; MAPK1; RAC2; PTPN11 ; AKT2; IKBKB; PIK3CA; CREB1; SYK; NFKB2; CAMK2A; MAP3K14; PIK3CB; PIK3C3; MAPK8; BCL2L1; ABL1; MAPK3; ETS1; KRAS; MAPK13; RELA; PTPN6; MAPK9; EGR1; PIK3C2A; BTK; MAPK14; RAF1; IKBKG; RELB; MAP3K7; MAP2K2; AKT1; PIK3R1; CHUK; MAP2K1;
B Cell Receptor Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
Leukocyte Extravasation Signaling	NFKB1; CDC42; GSK3A; FRAP1; BCL6; BCL10; JUN; GSK3B; ATF4; AKT3; VAV3; RPS6KB1 ACTN4; CD44; PRKCE; ITGAM; ROCK1; CXCR4; CYBA; RAC1; RAP1A; PRKCZ; ROCK2; RAC2; PTPN11; MMP14; PIK3CA; PRKCI; PTK2; PIK3CB; CXCL12; PIK3C3; MAPK8; PRKD1; ABL1; MAPK10; CYBB; MAPK13; RHOA; PRKCD; MAPK9; SRC; PIK3C2A; BTK; MAPK14; NOX1; PXN; VIL2; VASP; ITGB1; MAP2K2; CTNND1; PIK3R1 CTNND1; CLDN1 CDC42; F11R; ITK; CRKL; VAV3; CTTN; PRKCA; MMP1; MMP9 ACTN4; ITGAM; ROCK1; ITGA5; RAC1; PTEN; RAP1A; TLN1; ARHGEF7; MAPKI; RAC2; CAPNS1; AKT2; CAPN2; PIK3CA; PTK2; PIK3CB; PIK3C3; MAPK8; CAV1; CAPN1; ABLI; MAPK3; ITGA1; KRAS; RHOA; SRC; PIK3C2A; ITGB7; PPP1CC; ILK; PXN; VASP; RAF1; FYN; ITGB1; MAP2K2; PAK4; AKT1; PIK3R1; TNK2; MAP2K1; PAK3; ITGB3; CDC42; RND3; ITGA2; CRKL; BRAF; GSK3B; AKT3 IRAK1; SOD2; MYD88; TRAF6; ELK1; MAPK1; PTPN11; AKT2; IKKBK; PIK3CA; FOS; NFKB2; MAP3K14; PIK3CB; MAPK8; RIPK1; MAPK3; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; FTL; NR3C1; TRAF2; SERPINE1; MAPK14; TNF; RAF1; PDK1; IKBK; RELB; MAP3K7; MAP2K2; AKT1; JAK2; PIK3R1; CHUK; STAT3; MAP2K1; NFKB1; FRAP1; CEBPB; JUN; AKT3; IL1R1; IL6
	ITGAM; ITGA5; RAC1; PTEN; PRKCZ; BCL2L11; MAPKI; RAC2; AKT2; EGFR; IKKBK; CBL; PIK3CA; CDKN1B; PTK2; NFKB2; BCL2; PIK3CB; BCL2L1; MAPK3; ITGA1; KRAS; ITGB7; ILK; PDGFRB; INSR; RAF1; IKBK; CASP9; CDKN1A; ITGB1; MAP2K2; AKT1; PIK3R1; CHUK; PDGFRA; PDPK1; MAP2K1; NFKB1; ITGB3; CDC42; CCND1; GSK3A; ITGA2; GSK3B; AKT3; FOXO1; CASP3; RPS6KB1
	PTEN; EP300; BBC3; PCAF; FASN; BRCAT; GADD45A; BIRC5; AKT2; PIK3CA; CHEK1; TP53INP1; BCL2; PIK3CB; PIK3C3; MAPK8;
Integrin Signaling	
Acute Phase Response Signaling	
PTEN Signaling	
p53 Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
Aryl Hydrocarbon Receptor Signaling	THBS1; ATR; BCL2L1; E2F1; PMAIP1; CHEK2; TNFRSF10B; TP73; RBI; HDAC9; CDK2; PIK3C2A; MAPK14; TP53; LRDD; CDKN1A; HIPK2; AKT1; PIK3R1; RRM213; APAF1; CTNND1; SIRT1; CCND1; PRKDC; ATM; SFN; CDKN2A; JUN; SNAI2; GSK3B; BAX; AKT3 HSPB1; EP300; FASN; TGM2; RXRA; MAPK1; NQO1; NCOR2; SP1; ARNT; CDKN1B; FOS; CHEK1; SMARCA4; NFKB2; MAPK8; ALDH1A1; ATR; E2F1; MAPK3; NRIP1; CHEK2; RELA; TP73; GSTP1; RB1; SRC; CDK2; AHR; NFE2L2; NCOA3; TP53; TNF; CDKN1A; NCOA2; APAF1; NFKB1; CCND1 ATM; ESR1; CDKN2A; MYC; JUN; ESR2; BAX; IL6; CYP1B1; HSP90AA1 PRKCE; EP300; PRKCZ; RXRA; MAPK1; NQO1; NCOR2; PIK3CA; ARNT; PRKCI; NFKB2; CAMK2A; PIK3CB; PPP2R1A; PIK3C3; MAPK8; PRKD1; ALDH1A1; MAPK3; NRIP1; KRAS; MAPK13; PRKCD; GSTP1; MAPK9; NOS2A; ABCB1; AHR; PPP2CA; FTL; NFE2L2; PIK3C2A; PPARGC1A; MAPK14; TNF; RAF1; CREBBP; MAP2K2; PIK3R1; PPP2R5C; MAP2K1; NFKB1; KEAP1; PRKCA; EIF2AK3; IL6; CYP1B1; HSP90AA1 PRKCE; IRAK1; PRKAA2; EIF2AK2; RAC1; ELK1; GRK6; MAPK1; GADD45A; RAC2; PLK1; AKT2; PIK3CA; FADD; CDK8; PIK3CB; PIK3C3; MAPK8; RIPK1; GNB2L1; IRS1; MAPK3; MAPK10; DAXX; KRAS; PRKCD; PRKAA1; MARK9; CDK2; PIM1; PIK3C2A; TRAF2; TP53; LCK; MAP3K7; DYRK1A; MAP2K2; PIK3R1; MAP2K1; PAK3; CDC42; JUN; TTK; CSNK1A1; CRKL; BRAF; SGK PRKAA2; EP300; INS; SMAD2; TRAF6; PPARA; FASN; RXRA; MAPK1; SMAD3; GNAS; IKKBK; NCOR2; ABCA1; GNAQ; NFKB2; MAP3K14; STAT5B; MAPK8; IRS1; MAPK3; KRAS; RELA; PRKAA1; PPARGC1A; NCOA3; MAPK14; INSR; RAFT; IKBK; RELB; MAP3K7; CREBBP; MAP2K2; JAK2; CHUK; MAP2K1; NFKB1;
Xenobiotic Metabolism Signaling	
SAPK/JNK Signaling	
PPAr/RXR Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
NF- κ B Signaling	TGFB1; SMAD4; JUN; IL1R1; PRKCA; IL6; HSP90AA1; ADIPOQ IRAK1; EIF2AK2; EP300; INS; MYD88; PRKCZ; TRAF6; TBK1; AKT2; EGFR IKKBK; PIK3CA; BTRC; NFKB2; MAP3K14; PIK3CB; PIK3C3; MAPK8; RIPK1; HDAC2; KRAS; RELA; PIK3C2A; TRAF2; TLR4; PDGFRB; TNF; INSR; LCK; IKKBK; RELB; MAP3K7; CREBBP; AKT1; PIK3R1; CHUK; PDGFRA; NFKB1; TLR2; BCL10; GSK3B; AKT3; TNFAIP3; IL1R1
	ERBB4; PRKCE; ITGAM; ITGA5; PTEN; PRKCZ; ELK1; MAPK1; PTPN11; AKT2; EGFR; ERBB2; PRKCI; CDKN1B; STAT5B; PRKD1; MAPK3; ITGA1; KRAS; PRKCD; STAT5A; SRC; ITGB7; RAF1; ITGB1; MAP2K2; ADAM 17; AKT1; PIK3R1; PDPK1; MAP2K1; ITGB3; EREG; FRAP1; PSEN1; ITGA2; MYC; NRG1; CRKL; AKT3; PRKCA; HSP90AA1; RPS6KB1
Wnt & Beta catenin Signaling	CD44; EP300; LRP6; DVL3; CSNK1E; GJA1; SMO; AKT2; PIN1; CDH1; BTRC; GNAQ; MARK2; PPP2R1A; WNT11; SRC; DKK1; PPP2CA; SOX6; SFRP2; ILK; LEF1; SOX9; TP53; MAP3K7; CREBBP; TCF7L2; AKT1; PPP2R5C; WNT5A; LRP5; CTNBNB1; TGFB1; CCND1; GSK3A; DVL1; APC; CDKN2A; MYC; CSNK1A1; GSK3B; AKT3; SOX2
Insulin Receptor Signaling	PTEN; INS; EIF4E; PTPN1; PRKCZ; MAPK1; TSC1; PTPN11; AKT2; CBL; PIK3CA; PRKCI; PIK3CB; PIK3C3; MAPK8; IRS1; MAPK3; TSC2; KRAS; EIF4EBP1; SLC2A4; PIK3C2A; PPP1CC; INSR; RAF1; FYN; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; PDPK1; MAP2K1; GSK3A; FRAP1; CRKL; GSK3B; AKT3; FOXO1; SGK; RPS6KB1
	HSPB1; TRAF6; MAPKAPK2; ELK1; MAPK1; PTPN11; IKKBK; FOS; NFKB2; MAP3K14; MAPK8; MAPK3; MAPK10; IL6ST; KRAS; MAPK13; IL6R; RELA; SOCS1; MAPK9; ABCB1; TRAF2; MAPK14; TNF; RAF1; IKKBK; RELB; MAP3K7; MAP2K2; IL8; JAK2; CHUK; STAT3; MAP2K1; NFKB1; CEBPB; JUN; IL1R1; SRF; IL6
Hepatic Cholestasis	PRKCE; IRAK1; INS; MYD88; PRKCZ; TRAF6; PPARA; RXRA; IKKBK; PRKCI; NFKB2; MAP3K14; MAPK8;

TABLE C-continued

CELLULAR FUNCTION	GENES
IGF-1 Signaling	PRKD1; MAPK10; RELA; PRKCD; MAPK9; ABCB1; TRAF2; TLR4; TNF; INSR; IKKBK; RELB; MAP3K7; IL8; CHUK; NR1H2; TJP2; NFKI31; ESR1; SREBF1; FGFR4; JUN; IL1R1; PRKCA; IL6 IGF1; PRKCZ; ELK1; MAPK1; PTPN11; NEDD4; AKT2; PIK3CA; PRKC1; PTK2; FOS; PIK3CB; PIK3C3; MAPK8; IGF1R; IRS1; MAPK3; IGFBP7; KRAS; PIK3C2A; YWHAZ; PXN; RAF1; CASP9; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; IGFBP2; SFN; JUN; CYR61; AKT3; FOXO1; SRF; CTGF; RPS6KB1
	PRKCE; EP300; SOD2; PRKCZ; MAPK1; SQSTM1; NQO1; PIK3CA; PRKCI; FOS; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; KRAS; PRKCD; GSTP1; MAPK9; FTL; NFE2L2; PIK3C2A; MAPK14; RAF1; MAP3K7; CREBBP; MAP2K2; AKT1; PIK3R1; MAP2K1; PPIB; JUN; KEAP1; GSK3B; ATF4; PRKCA; EIF2AK3; HSP90AA1
NRF2-mediated Oxidative Stress Response	EDN1; IGF1; KDR; FLT1; SMAD2; FGFR1; MET; PGF; SMAD3; EGFR; FAS; CSF1; NFKB2; BCL2; MYH9; IGHF1R; IL6R; RELA; TLR4; PDGFRB; TNF; RELB; IL8; PDGFRA; NFKB1; TGFB1; SMAD4; VEGFA; BAX; IL1R1; CCL2; HGF; MMP1; STAT1; IL6; CTGF; MMP9 EP300; INS; TRAF6; PPARA; RXRA; MAPK1; IKKBK; NCOR2; FOS; NFKB2; MAP3K14; STAT5B; MAPK3; NRIP1; KRAS; PPARG; RELA; STAT5A; TRAF2; PPARGC1A; PDGFRB; TNF; INSR; RAF1; IKKBK; RELB; MAP3K7- CREBBP; MAP2K2; CHUK; PDGFRA; MAP2K1; NFKB1; JUN; IL1R1; HSP90AA1
Hepatic Fibrosis/ Hepatic Stellate Cell Activation	PRKCE; RAC1; PRKCZ; LYN; MAPK1; RAC2; PTPN11; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; MAPK8; PRKD1; MAPK3; MAPK10; KRAS; MAPK13; PRKCD; MAPK9; PIK3C2A; BTK; MAPK14; TNF; RAF1; FYN; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; AKT3; VAV3; PRKCA
PPAR Signaling	PRKCE; RAP1A; RGS16; MAPK1; GNAS; AKT2; IKKBK; PIK3CA; CREB1; GNAQ; NFKB2; CAMK2A; PIK3CB; PIK3C3; MAPK3; KRAS; RELA; SRC; PIK3C2A; RAF1;
Fc Epsilon RI Signaling	
G-Protein Coupled	
Receptor Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
Inositol Phosphate Metabolism	IKBK; RELB; FYN; MAP2K2; AKT1; PIK3R1; CHUK; PDPK1; STAT3; MAP2K1; NFKB1; BRAF; ATF4; AKT3; PRKCA PRKCE; IRAK1; PRKAA2; EIF2AK2; PTEN; GRK6; MAPK1; PLK1; AKT2; PIK3CA; CDK8; PIK3CB; PIK3C3; MAPK8; MAPK3; PRKCD; PRKAA1; MAPK9; CDK2; PIM1; PIK3C2A; DYRK1A; MAP2K2; PIP5K1A; PIK3R1; MAP2K1; PAK3; ATM; TTK; CSNK1A1; BRAF; SGK EIF2AK2; ELK1; ABL2; MAPK1; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; CAV1; ABL1; MAPK3; KRAS; SRC; PIK3C2A; PDGFRB; RAF1; MAP2K2; JAK1; JAK2; PIK3R1; PDGFRA; STAT3; SPHK1; MAP2K1; MYC; JUN; CRKL; PRKCA; SRF; STAT1; SPHK2
PDGF Signaling	ACTN4; ROCK1; KDR; FLT1; ROCK2; MAPK1; PGF; AKT2; PIK3CA; ARNT; PTK2; BCL2; PIK3CB; PIK3C3; BCL2L1; MAPK3; KRAS; HIF1A; NOS3; PIK3C2A; PXN; RAF1; MAP2K2; ELAVL1; AKT1; PIK3R1; MAP2K1; SFN; VEGFA; AKT3; FOXO1; PRKCA PRKCE; RAC1; PRKCZ; MAPK1; RAC2; PTPN11; KIR2DL3; AKT2; PIK3CA; SYK; PRKCI; PIK3CB; PIK3C3; PRKD1; MAPK3; KRAS; PRKCD; PTPN6; PIK3C2A; LCK; RAF1; FYN; MAP2K2; PAK4; AKT1; PIK3R1; MAP2K1; PAK3; AKT3; VAV3; PRKCA
VEGF Signaling	HDAC4; SMAD3; SUV39H1; HDAC5; CDKN1B; BTRC; ATR; ABL1; E2F1; HDAC2; HDAC7A; RBL1; HDAC11; HDAC9; CDK2; E2F2; HDAC3; TP53; CDKN1A; CCND1; E2F4; ATM; RBL2; SMAD4; CDKN2A; MYC; NRG1; GSK3B; RBL1; HDAC6
Natural Killer Cell Signaling	RAC1; ELK1; MAPK1; IKKB; CBL; PIK3CA; FOS; NFKB2; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; RELA; PIK3C2A; BTK; LCK; RAF1; IKKBK; RELB; FYN;
Cell Cycle: G1/S	
Checkpoint Regulation	
T Cell Receptor Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
Death Receptor Signaling	MAP2K2; PIK3R1; CHUK; MAP2K1; NFKB1; ITK; BCL10; JUN; VAV3 CRADD; HSPB1; BID; BIRC4; TBK1; IKKBK; FADD; FAS; NFKB2; BCL2; MAP3K14; MAPK8; RIPK1; CASP8; DAXX; TNFRSF10B; RELA; TRAF2; TNF; IKKBK; RELB; CASP9; CHUK; APAF1; NFKB1; CASP2; BIRC2; CASP3; BIRC3
FGF Signaling	RAC1; FGFR1; MET; MAPKAPK2; MAPK1; PTPN11; AKT2; PIK3CA; CREB1; PIK3CB; PIK3C3; MAPK8; MAPK3; MAPK13; PTPN6; PIK3C2A; MAPK14; RAF1; AKT1; PIK3R1; STAT3; MAP2K1; FGFR4; CRKL; ATF4; AKT3; PRKCA; HCF LYN; ELK1; MAPK1; PTPN11; AKT2; PIK3CA; CAMK2A; STAT5B; PIK3CB; PIK3C3; GNB2L1; BCL2L1; MAPK3; ETS1; KRAS; RUNX1; PIM1; PIK3C2A; RAF1; MAP2K2; AKT1; JAK2; PIK3R1; STAT3; MAP2K1; CCND1; AKT3; STAT1
GM-CSF Signaling	BID; IGF1; RAC1; BIRC4; PGF; CAPNS1; CAPN2; PIK3CA; BCL2; PIK3CB; PIK30; BCL2L1; CAPN1; PIK3C2A; TP53; CASP9; PIK3R1; RAB5A; CASP1; APAF1; VEGFA; BIRC2; BAX; AKT3; CASP3; BIRC3 PTPNI; MAPK1; PTPN11; AKT2; PIK3CA; STAT5B; PIK3CB; PIK3C3; MAPK3; KRAS; SOCS1; STAT5A; PTPN6; PIK3C2A; RAF1; CDKN1A; MAP2K2; JAK1; AKT1; JAK2; PIK3R1; STAT3; MAP2K1; FRAP1; AKT3; STAT1
Amyotrophic Lateral Sclerosis Signaling	PRKCE; IRAK1; PRKAA2; EIF2AK2; GRK6; MAPK1; PLK1; AKT2; CDK8; MAPK8; MAPK3; PRKCD; PRKAA1; PBEF1; MAPK9; CDK2; PIM1; DYRK1A; MAP2K2; MAP2K1; PAK3; NT5E; TTK; CSNK1A1; BRAF; SGK
JAK/ Stat Signaling	
Nicotinate and Nicotinamide Metabolism	

TABLE C-continued

CELLULAR FUNCTION	GENES
Chemokine Signaling	CXCR4; ROCK2; MAPK1; PTK2; FOS; CFL1; GNAQ; CAMK2A; CXCL12.; MAPK8; MAPK3; KRAS; MAPK13; RHOA; CCR3; SRC; PPP1CC; MAPK14; NOX1; RAF1; MAP2K2; MAP2K1; JUN; CCL2; PRKCA ELK1; MAPK1; PTPN11; AKT2; PIK3CA; SYK; FOS; STAT5B; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; SOCS1; STAT5A; PIK3C2A; LCK; RAF1; MAP2K2; JAK1; AKT1; PIK3R1; MAP2K1; JUN; AKT3
IL-2 Signaling	PRKCE; IGF1; PRKCZ; PRDX6; LYN; MAPK1; GNAS; PRKCI; GNAQ; PPP2R1A; IGFIR; PRKDI; MAPK3; KRAS; GRN; PRKCD; NOS3; NOS2A; PPP2CA; YWHAZ; RAF1; MAP2K2; PPP2R5C; MAP2K1; PRKCA
Synaptic Long Term Depression	TAF4B; EP300; CARM1; PCAF; MAPK1; NCOR2; SMARCA4; MAPK3; NRIP1; KRAS; SRC; NR3C1; HDAC3; PPARGC1A; RBM9; NCOA3; RAF1; CREBBP; MAP2K2; NCOA2; MAP2K1; PRKDC; ESR1; ESR2
Estrogen Receptor Signaling	TRAF6; SMURF1; BIRC4; BRCA1; UCHL1; NEDD4; CBL; UBE2L; BTRC; HSPA5; USP7; USP10; FBXW7; USP9X; STUB1; USP22; B2M; BIRC2; PARK2; USP8; USP1; VHL; HSP90AA1; BIRC3
Protein Ubiquitination Pathway	TRAF6; CCR1; ELK1; IKBKB; SP1; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; MAPK14; TNF; IKBKG; RELB; MAP3K7; JAK1; CHUK; STAT3; NFKB1; JUN; IL1R1; IL6 PRKCE; EP300; PRKCZ; RXRA; GADD45A; HES1; NCOR2; SP1; PRKCI; CDKN1B; PRKDI; PRKCD; RUNX2; KLF4; YY1; NCOA3; CDKN1A; NCOA2; SPP1; LRP5; CEBPB; FOXO1; PRKCA
IL-10 Signaling	
VDR/ RXR Activation	

TABLE C-continued

CELLULAR FUNCTION	GENES
TGF- beta Signaling	EP300; SMAD2; SMURF1; MAPK1; SMAD3; SMAD1; FOS; MAPK8; MAPK3; KRAS; MAPK9; RUNX2.; SERPINE1; RAF1; MAP3K7; CREBBP; MAP2K2; MAP2K1; TGFBRI; SMAD4; JUN; SMAD5
Toll- like Receptor Signaling	IRAK1; EIF2AK2; MYD88; TRAF6; PPARA; ELK1; IKBKB; FOS; NFKB2; MAP3K14; MAPK8; MAPK13; RELA; TLR4; MAPK14; IKBKG; RELB; MAP3K7; CHUK; NFKB1; TLR2; JUN HSPB1; IRAK1; TRAF6; MAPK MAPKAPK2; ELK1; FADD; FAS; CREB1; DDIT3; RPS6KA4; DAXX; MAPK13; TRAF2; MAPK 14; TNF; MAP3K7; TGFBRI; MYC; ATF4; IL1R1 ; SRF; STAT1
p38 Signaling	NTRK2; MAPK1; PTPN11 ; PIK3CA; CREB1; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; KRAS; PIK3C2A; RAF1; MAP2K2; AKT1; PIK3R1; PDPK1; MAP2K1; CDC42; JUN; ATF4 INS; PPARA; FASN; RXRA; AKT2; SDC1; MAPK8; APOB; MAPK10; PPARG; MTTT; MAPK9; PPARGC1A; TNF; CREBBP; AKT1; SREBF1; FGFR4; AKT3; FOXO1 PRKCE; RAP1A; EP300; PRKCZ; MAPK1; CREB1; PRKCI; GNAQ; CAMK2A; PRKDI; MAPK3; KRAS; PRKCD; PPP1CC; RAF1; CREBBP; MAP2K2; MAP2K1; ATF4; PRKCA RAP1A; EP300; HDAC4; MAPK1; HDAC5; CREB1; CAMK2A; MYH9; MAPK3; HDAC2; HDAC7A; HDAC11; HDAC9; HDAC3; CREBBP; CALR; CAMKK2; ATF4; HDAC6
Neurotrophin/ TRK Signaling	ELK1; MAPK1; EGFR; PIK3CA; FOS; PIK3CB; PIK3C3; MAPK8; MAPK3; PIK3C2A; RAF1; JAK1; PIK3R1; STAT3; MAP2K1; JUN; PRKCA; SRF; STAT1
FXR/ RXR Activation	
Synaptic Long Term Potentiation	
Calcium Signaling	
EGF Signaling	

TABLE C-continued

CELLULAR FUNCTION	GENES
Hypoxia Signaling in the Cardiovascular System	EDN1; PTEN; EP300; NQO1; UBE2I; CREB1; ARNT; HIF1A; SLC2A4; NOS3; TP53; LDHA; AKT1; ATM; VEGFA; JUN; ATF4; VHL; HSP90AA1
LPS/ IL-1 Mediated. Inhibition of RXR Function	IRAK1; MYD88; TRAF6; PPARA; RXRA; ABCA1; MAPK8; ALDH1A1; GSTP1; MAPK9; ABCB1; TRAF2; TLR4; TNF; MAP3K7; NR1H2; SREBF1; JUN; IL1R1
LXR/ RXR Activation	FASN; RXRA; NCOR2; ABCA1.; NFKB2; IRF3; RELA; NOS2A; TLR4; TNF; RELB; LDLR; NR1H2; NFKB1; SREBF1; IL1R1; CCL2; IL6; MMP9
Amyloid Processing	PRKCE; CSNK1E; MAPK1; CAPNS1; AKT2; CAPN2; CAPN1; MAPK3; MAPK13; MAPT; MAPK14; AKT1; PSEN1; CSNK1A1; GSK3B; AKT3; APP
1L-4 Signaling	AKT2; PIK3CA; PIK3CB; PIK3C3; IRS1; KRAS; SOCS1; PTPN6; NR3C1; PIK3C2A; JAK1; AKT1; JAK2; PIK3R1; FRAP1; AKT3; RPS6KB1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation Nitric Oxide Signaling in the Cardiovascular System	EP300; PCAF; BRCA1; GADD45A; PLK1; BTRC; CHEK1; ATR; CHEK2; YWHAZ; TP53; CDKN1A; PRKDC; ATM; SFN; CDKN2A KDR; FLT1; PGF; AKT2; PIK3CA; PIK3CB; PIK3C3; CAV1; PRKCD; NOS3; PIK3C2A; AKT1; PIK3R1;
Purine Metabolism	VEGFA.; AKT3; HSP90AA1 NME2; SMARCA4; MYH9; RRM2; ADAR.; EIF2AK4 PKM2; ENTPD1; RAD51; RRM2B; TJP2; .RAD51C; NT5E; POLD1; NME1
cAMP- mediated Signaling	RAP1A; MAPK1; GNAS; CREB1; CAMK2A; MAPK3; SRC; RAH; MAP2K2; STAT3; MAP2K1; BRAF; ATF4
Mitochondrial Dysfunction	SOD2; MAPK8; CASP8; MAPK10; MAPK9 CASP9; PARK7; PSEN1; PARK2; APP; CASP3
Notch Signaling	HES1; JAG1; NUMB; NOTCH4; ADAM17; NOTCH2; PSEN1; NOTCH3; NOTCH1; DLL4

TABLE C-continued

CELLULAR FUNCTION	GENES
Endoplasmic Reticulum Stress Pathway Pyrimidine Metabolism	HSPA5; MAPK8; XBP1; TRAF2; ATF6; CASP9; ATF4; EIF2AK3; CASP3 NME2; AICDA; RRM2; EIF2AK4; ENTPD1; RRM2B; NT5E; POLD1; NME1
Parkinson's Signaling	UCHL1; MAPK8; MAPK13; MAPK14; CASP9; PARK7; PARK2; CASP3
Cardiac & Beta Adrenergic Signaling Glycolysis/ Gluconeogenesis	GNAS; GNAQ; PPP2R1A; GNB2L1; PPP2CA; PPP1CC; PPP2R5C HK2; GCK; GPI; ALDH1A1; PKM2; LDHA; HK1
Interferon Signaling Sonic Hedgehog Signaling Glycero- phospholipid. Metabolism Phospholipid Degradation Tryptophan Metabolism Lysine Degradation Nucleotide Excision Repair Pathway Starch and Sucrose Metabolism Aminosugars Metabolism Arachidonic Acid Metabolism Circadian Rhythm Signaling Coagulation System Dopamine Receptor Signaling Glutathione Metabolism Glycerolipid Metabolism Linoleic Acid Metabolism Methionine Metabolism. Pyruvate Metabolism Arginine and Proline Metabolism Eicosanoid Signaling Fructose and Mannose Metabolism Galactose Metabolism Stilbene, Cournarine and Lignin Biosynthesis	IRF1; SOCS1; JAK1; JAK2; IFITM1; STAT1; IFIT3 ARRB2; SMO; GLI2; DYRK1A; GLI1; GSK3B; DYRK1B PLD1; GRN; GPAM; YWHAZ; SPHK1; SPHK2 PRDX6; PLD1; GRN; YWHAZ; SPHK1; SPHK2 SIAH2; PRMT5; NEDD4; ALDH1A1; CYP1B1; SIAH1 SUV39H1; EHMT2; NSD1; SETD7; PPP2R5C ERCC5; ERCC4; XPA; XPC; ERCC1
	UCHL1; HK2; GCK; GPI; HK1
	NQO1; HK2; GCK; HK1 PRDX6; GRN; YWHAZ; CYP1B1 CSNK1E; CREB1; ATF4; NR1D1
	BDKRB1; F2R; SERPINE1; F3 PPP2R1A; PPP2CA; PPP1CC; PPP2R5C
	IDH2; GSTP1; ANPEP; IDH1 ALDH1A1; GPAM; SPHK1; SPHK2 PRDX6; GRN; YWHAZ; CYP1B1
	DNMT1; DNMT3B; AHCY; DNMT3A GLO1; ALDH1A1; PKM2; LDHA ALDH1A1; NOS3; NOS2A
	PRDX6; GRN; YWHAZ HK2; GCK; HK1
	HK2; GCK; HK1 PRDX6; PRDX1; TYR

TABLE C-continued

CELLULAR FUNCTION	GENES
Antigen Presentation Pathway	CALR; B2M
Biosynthesis of Steroids	NQO1; DHCR7
Butanoate Metabolism	ALDH1A1; NLGN1
Citrate Cycle	IDH2; IDH1
Fatty Acid Metabolism	ALDH1A1; CYP11B1
Glycerophospholipid Metabolism	PRDX6; CHKA
Histidine Metabolism	PRMT5; ALDH1A1
Inositol Metabolism	ERO1L; APEX1
Metabolism of Xenobiotics	GSTP1; CYP11B1
by Cytochrome p450	
Methane Metabolism	PRDX6; PRDX1
Phenylalanine Metabolism	PRDX6; PRDX1
Propanoate Metabolism	ALDH1A1; LDHA
Selenoamino Acid Metabolism	PRMT5; AHCY
Sphingolipid Metabolism	SPHK1; SPHK2
Aminophosphonate Metabolism	PRMT5
Androgen and Estrogen Metabolism	PRMT5
Ascorbate and Aldarate Metabolism	ALDH1A1
Bile Acid Biosynthesis	ALDH1A1
Cysteine Metabolism	LDHA
Fatty Acid Biosynthesis	FASN
Glutamate Receptor Signaling	GNB2L1
NRF2-mediated Oxidative Stress Response	PRDX1
Pentose Phosphate Pathway	GPI
Pentose and Glucuronate interconversions	UCHL1
Retinol Metabolism	ALDH1A1
Riboflavin Metabolism	TYR
Tyrosine Metabolism	PRMT5; TYR
Ubiquinone Biosynthesis	PRMT5
Valine, Leucine and Isoleucine Degradation	ALDH1A1
Glycine, Serine and Threonine Metabolism	CHKA
Lysine Degradation	ALDH1A1
Pain/Taste	TRPM5; TRPA1
Pain	TRPM7; TRPC5; TRPC6; TRPC1; Cnr1; cnr2; Grk2; Trp1; Pomc; Cgrp; Crf; Pka; Era; Nr2b; TRPM5; Prkaca; Prkacb; Prkar1a; Prkar2a
Mitochondrial Function	AIF; CytC; SMAC (Diablo); Aifm-1; Aifm-2
Developmental Neurology	BMP-4; Chordin (Chrd); Noggin (Nog); WNT (Wnt2; Wnt2b; Wnt3a; Wnt4; Wnt5a; Wnt6; Wnt7b; Wnt8b; Wnt9a; Wnt9b; Wnt10a; Wnt10b; Wnt16); beta-catenin; Dkk-1; Frizzled related proteins; Otx-2; Gbx2; FGF-8; Reelin; Dab1; unc-86 (Pou4f1 or Brn3a); Numb; Reln

[0126] Embodiments of the invention also relate to methods and compositions related to knocking out genes, amplifying genes and repairing particular mutations associated with DNA repeat instability and neurological disorders (Robert D. Wells, Tetsuo Ashizawa, Genetic Instabilities and Neurological Diseases, Second Edition, Academic Press, Oct. 13, 2011—Medical). Specific aspects of tandem repeat

sequences have been found to be responsible for more than twenty human diseases (New insights into repeat instability: role of RNA*DNA hybrids. Melvor E I, Polak U. Napierala M. RNA Biol. 2010 September-October; 7(5):551-8). The CRISPR-Cas system may be harnessed to correct these defects of genomic instability.

[0127] A further aspect of the invention relates to utilizing the CRISPR-Cas system for correcting defects in the EMP2A and EMP2B genes that have been identified to be associated with Lafora disease. Lafora disease is an autosomal recessive condition which is characterized by progressive myoclonus epilepsy which may start as epileptic seizures in adolescence. A few cases of the disease may be caused by mutations in genes yet to be identified. The disease causes seizures, muscle spasms, difficulty walking, dementia, and eventually death. There is currently no therapy that has proven effective against disease progression. Other genetic abnormalities associated with epilepsy may also be targeted by the CRISPR-Cas system and the underlying genetics is further described in Genetics of Epilepsy and Genetic Epilepsies, edited by Giuliano Avanzini, Jeffrey L. Noebels, Mariani Foundation Paediatric Neurology; 20; 2009).

[0128] In yet another aspect of the invention, the CRISPR-Cas system may be used to correct ocular defects that arise from several genetic mutations further described in Genetic Diseases of the Eye, Second Edition, edited by Elias I. Traboulsi, Oxford University Press, 2012.

[0129] Several further aspects of the invention relate to correcting defects associated with a wide range of genetic diseases which are further described on the website of the National Institutes of Health under the topic subsection Genetic Disorders. The genetic brain diseases may include but are not limited to Adrenoleukodystrophy, Agenesis of the Corpus Callosum, Aicardi Syndrome, Alpers' Disease, Alzheimer's Disease, Barth Syndrome, Batten Disease, CADASIL, Cerebellar Degeneration, Fabry's Disease, Gerstmann-Straussler-Scheinker Disease, Huntington's Disease and other Triplet Repeat Disorders, Leigh's Disease, Lesch-Nyhan Syndrome, Menkes Disease, Mitochondrial Myopathies and NINDS Colpocephaly. These diseases are further described on the website of the National Institutes of Health under the subsection Genetic Brain Disorders.

[0130] In some embodiments, the condition may be neoplasia. In some embodiments, where the condition is neoplasia, the genes to be targeted are any of those listed in Table A (in this case PTEN and so forth). In some embodiments, the condition may be Age-related Macular Degeneration. In some embodiments, the condition may be a Schizophrenic Disorder. In some embodiments, the condition may be a Trinucleotide Repeat Disorder. In some embodiments, the condition may be Fragile X Syndrome. In some embodiments, the condition may be a Secretase Related Disorder. In some embodiments, the condition may be a Prion—related disorder. In some embodiments, the condition may be ALS. In some embodiments, the condition may be inflammation. In some embodiments, the condition may be a drug addiction. In some embodiments, the condition may be Autism. In some embodiments, the condition may be Alzheimer's Disease. In some embodiments, the condition may be inflammation. In some embodiments, the condition may be Parkinson's Disease.

[0131] Examples of proteins associated with Parkinson's disease include but are not limited to α -synuclein, DJ-1, LRRK2, PINK1, Parkin, UCHL1, Synphilin-1, and NURR1.

[0132] Examples of addiction-related proteins may include ABAT for example.

[0133] Examples of inflammation-related proteins may include the monocyte chemoattractant protein-1 (MCP1) encoded by the *Ccr2* gene, the C-C chemokine receptor type 5 (CCR5) encoded by the *Ccr5* gene, the IgG receptor IIB (FCGR2b, also termed CD32) encoded by the *Fcgr2b* gene, or the Fc epsilon R1g (FCER1g) protein encoded by the *Fcer1g* gene, for example.

[0134] Examples of cardiovascular diseases associated proteins may include IL1B (interleukin 1, beta), XDH (xanthine dehydrogenase), TP53 (tumor protein p53), PTGIS (prostaglandin I2 (prostacyclin) synthase), MB (myoglobin), IL4 (interleukin 4), ANGPT1 (angiopoietin 1), ABCG8 (ATP-binding cassette, sub-family G (WHITE), member 8), or CTSK (cathepsin K), for example.

[0135] Examples of Alzheimer's disease associated proteins may include the very low density lipoprotein receptor protein (VLDLR) encoded by the *VLDLR* gene, the ubiquitin-like modifier activating enzyme 1 (UBA1) encoded by the *UBA1* gene, or the NEDD8-activating enzyme E1 catalytic subunit protein (UBE1C) encoded by the *UBA3* gene, for example.

[0136] Examples of proteins associated with Autism Spectrum Disorder may include the benzodiazepine receptor (peripheral) associated protein 1 (BZRAP1) encoded by the *BZRAP1* gene, the AF4/FMR2 family member 2 protein (AFF2) encoded by the *AFF2* gene (also termed MFR2), the fragile X mental retardation autosomal homolog 1 protein (FXR1) encoded by the *FXR1* gene, or the fragile X mental retardation autosomal homolog 2 protein (FXR2) encoded by the *FXR2* gene, for example.

[0137] Examples of proteins associated with Macular Degeneration may include the ATP-binding cassette, sub-family A (ABC1) member 4 protein (ABCA4) encoded by the *ABCR* gene, the apolipoprotein E protein (APOE) encoded by the *APOE* gene, or the chemokine (C-C motif) Ligand 2 protein (CCL2) encoded by the *CCL2* gene, for example.

[0138] Examples of proteins associated with Schizophrenia may include NRG1, ErbB4, CPLX1, TPH1, TPH2, NRXN1, GSK3A, BDNF, DISC1, GSK3B, and combinations thereof.

[0139] Examples of proteins involved in tumor suppression may include ATM (ataxia telangiectasia mutated), ATR (ataxia telangiectasia and Rad3 related), EGFR (epidermal growth factor receptor), ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2), ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3), ERBB4 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 4), Notch 1, Notch2, Notch 3, or Notch 4, for example.

[0140] Examples of proteins associated with a secretase disorder may include PSENEN (presenilin enhancer 2 homolog (*C. elegans*)), CTSB (cathepsin B), PSEN1 (presenilin 1), APP (amyloid beta (A4) precursor protein), APL1B (anterior pharynx defective 1 homolog B (*C. elegans*)), PSEN2 (presenilin 2 (Alzheimer disease 4)), or BACE1 (beta-site APP-cleaving enzyme 1), for example.

[0141] Examples of proteins associated with Amyotrophic Lateral Sclerosis may include SOD1 (superoxide dismutase 1), ALS2 (amyotrophic lateral sclerosis 2), FUS (fused in sarcoma), TARDBP (TAR DNA binding protein), VAGFA (vascular endothelial growth factor A), VAGFB (vascular endothelial growth factor B), and VAGFC (vascular endothelial growth factor C), and any combination thereof.

[0142] Examples of proteins associated with prion diseases may include SOD1 (superoxide dismutase 1), ALS2 (amyotrophic lateral sclerosis 2), FUS (fused in sarcoma), TARDBP (TAR DNA binding protein), VAGFA (vascular endothelial growth factor A), VAGFB (vascular endothelial growth factor B), and VAGFC (vascular endothelial growth factor C), and any combination thereof.

[0143] Examples of proteins related to neurodegenerative conditions in prion disorders may include A2M (Alpha-2-Macroglobulin), AATF (Apoptosis antagonizing transcription factor), ACPP (Acid phosphatase prostate), ACTA2 (Actin alpha 2 smooth muscle aorta), ADAM22 (ADAM metalloproteinase domain), ADORA3 (Adenosine A3 receptor), or ADRA1D (Alpha-1D adrenergic receptor for Alpha-1D adrenoreceptor), for example.

[0144] Examples of proteins associated with Immunodeficiency may include A2M [alpha-2-macroglobulin]; AANAT [arylalkylamine N-acetyltransferase]; ABCA1 [ATP-binding cassette, sub-family A (ABC1), member 1]; ABCA2 [ATP-binding cassette, sub-family A (ABC1), member 2]; or ABCA3 [ATP-binding cassette, sub-family A (ABC1), member 3]; for example.

[0145] Examples of proteins associated with Trinucleotide Repeat Disorders include AR (androgen receptor), FMR1 (fragile X mental retardation 1), HTT (huntingtin), or DMPK (dystrophin myotonic-protein kinase), FXN (frataxin), ATXN2 (ataxin 2), for example.

[0146] Examples of proteins associated with Neurotransmission Disorders include SST (somatostatin), NOS1 (nitric oxide synthase 1 (neuronal)), ADRA2A (adrenergic, alpha-2A-, receptor), ADRA2C (adrenergic, alpha-2C-, receptor), TACR1 (tachykinin receptor 1), or HTR2c (5-hydroxytryptamine (serotonin) receptor 2C), for example.

[0147] Examples of neurodevelopmental-associated sequences include A2BP1 [ataxin 2-binding protein 1], AADAT [aminoadipate aminotransferase], AANAT [arylalkylamine N-acetyltransferase], ABAT [4-aminobutyrate aminotransferase], ABCA1 [ATP-binding cassette, sub-family A (ABC1), member 1], or ABCA13 [ATP-binding cassette, sub-family A (ABC1), member 13], for example.

[0148] Further examples of preferred conditions treatable with the present system include may be selected from: Aicardi-Goutieres Syndrome; Alexander Disease; Allan-Herndon-Dudley Syndrome; POLG-Related Disorders; Alpha-Mannosidosis (Type II and III); Alström Syndrome; Angelman Syndrome; Ataxia-Telangiectasia; Neuronal Ceroid-Lipofuscinoses; Beta-Thalassemia; Bilateral Optic Atrophy and (Infantile) Optic Atrophy Type 1; Retinoblastoma (bilateral); Canavan Disease; Cerebrooculofacioskeletal Syndrome 1 [COFS 1]; Cerebrotendinous Xanthomatosis; Cornelia de Lange Syndrome; MAPT-Related Disorders; Genetic Prion Diseases; Dravet Syndrome; Early-Onset Familial Alzheimer Disease; Friedreich Ataxia [FRDA]; Fryns Syndrome; Fucosidosis; Fukuyama Congenital Muscular Dystrophy; Galactosialidosis; Gaucher Disease; Organic Acidemias; Hemophagocytic Lymphohistiocytosis; Hutchinson-Gilford Progeria Syndrome; Mucopolysaccharidosis 11; Infantile Free Sialic Acid Storage Disease; PLA2G6-Associated Neurodegeneration; Jervell and Lange-Nielsen Syndrome; Junctional Epidermolysis Bullosa; Huntington Disease; Krabbe Disease (Infantile); Mitochondrial DNA-Associated Leigh Syndrome and NARP; Lesch-Nyhan Syndrome; LIS1-Associated Lissencephaly; Lowe Syndrome; Maple Syrup Urine Disease; MECP2 Duplication

Syndrome; ATP7A-Related Copper Transport Disorders; LAMA2-Related Muscular Dystrophy; Arylsulfatase A Deficiency; Mucopolysaccharidosis Types I, II or III; Peroxisome Biogenesis Disorders, Zellweger Syndrome Spectrum; Neurodegeneration with Brain Iron Accumulation Disorders; Acid Sphingomyelinase Deficiency; Niemann-Pick Disease Type C; Glycine Encephalopathy; ARX-Related Disorders; Urea Cycle Disorders; COL1A1/2-Related Osteogenesis Imperfecta; Mitochondrial DNA Deletion Syndromes; PLP1-Related Disorders; Perry Syndrome; Phelan-McDermid Syndrome; Glycogen Storage Disease Type II (Pompe Disease) (Infantile); MAPT-Related Disorders; MECP2-Related Disorders; Rhizomelic Chondrodysplasia Punctata Type 1; Roberts Syndrome; Sandhoff Disease; Schindler Disease—Type 1; Adenosine Deaminase Deficiency; Smith-Lemli-Opitz Syndrome; Spinal Muscular Atrophy; Infantile-Onset Spinocerebellar Ataxia; Hexosaminidase A Deficiency; Thanatophoric Dysplasia Type 1; Collagen Type VI-Related Disorders; Usher Syndrome Type I; Congenital Muscular Dystrophy; Wolf-Hirschhorn Syndrome; Lysosomal Acid Lipase Deficiency; and Xeroderma Pigmentosum.

[0149] Chronic administration of protein therapeutics may elicit unacceptable immune responses to the specific protein. The immunogenicity of protein drugs can be ascribed to a few immunodominant helper T lymphocyte (HTL) epitopes. Reducing the MHC binding affinity of these HTL epitopes contained within these proteins can generate drugs with lower immunogenicity (Tangri S, et al. (“Rationally engineered therapeutic proteins with reduced immunogenicity” J Immunol. 2005 Mar. 15; 174(6):3187-96.) In the present invention, the immunogenicity of the CRISPR enzyme in particular may be reduced following the approach first set out in Tangri et al with respect to erythropoietin and subsequently developed. Accordingly, directed evolution or rational design may be used to reduce the immunogenicity of the CRISPR enzyme (for instance a Cas9) in the host species (human or other species).

[0150] In plants, pathogens are often host-specific. For example, *Fusarium oxysporum* f. sp. *lycopersici* causes tomato wilt but attacks only tomato, and *F. oxysporum* f. *dianthii* *Puccinia graminis* f. sp. *tritici* attacks only wheat. Plants have existing and induced defenses to resist most pathogens. Mutations and recombination events across plant generations lead to genetic variability that gives rise to susceptibility, especially as pathogens reproduce with more frequency than plants. In plants there can be non-host resistance, e.g., the host and pathogen are incompatible. There can also be Horizontal Resistance, e.g., partial resistance against all races of a pathogen, typically controlled by many genes and Vertical Resistance, e.g., complete resistance to some races of a pathogen but not to other races, typically controlled by a few genes. In a Gene-for-Gene level, plants and pathogens evolve together, and the genetic changes in one balance changes in other. Accordingly, using Natural Variability, breeders combine most useful genes for Yield, Quality, Uniformity, Hardiness, Resistance. The sources of resistance genes include native or foreign Varieties, Heirloom Varieties, Wild Plant Relatives, and Induced Mutations, e.g., treating plant material with mutagenic agents. Using the present invention, plant breeders are provided with a new tool to induce mutations. Accordingly, one skilled in the art can analyze the genome of sources of resistance genes, and in Varieties having desired characteristics or traits employ the present invention to

induce the rise of resistance genes, with more precision than previous mutagenic agents and hence accelerate and improve plant breeding programs.

[0151] As will be apparent, it is envisaged that the present system can be used to target any polynucleotide sequence of interest. Some examples of conditions or diseases that might be usefully treated using the present system are included in the Tables above and examples of genes currently associated with those conditions are also provided there. However, the genes exemplified are not exhaustive.

EXAMPLES

[0152] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1

CRISPR Complex Activity in the Nucleus of a Eukaryotic Cell

[0153] An example type II CRISPR system is the type 11 CRISPR locus from *Streptococcus pyogenes* SF370, which contains a cluster of four genes Cas9, Cas1, Cas2, and Csn1, as well as two non-coding RNA elements, tracrRNA and a characteristic array of repetitive sequences (direct repeats) interspaced by short stretches of non-repetitive sequences (spacers, about 30 bp each). In this system, targeted DNA double-strand break (DSB) is generated in four sequential steps (FIG. 2A). First, two non-coding RNAs, the pre-crRNA array and tracrRNA, are transcribed from the CRISPR locus. Second, tracrRNA hybridizes to the direct repeats of pre-crRNA, which is then processed into mature crRNAs containing individual spacer sequences. Third, the mature crRNA: tracrRNA complex directs Cas9 to the DNA target consisting of the protospacer and the corresponding PAM via heteroduplex formation between the spacer region of the crRNA and the protospacer DNA. Finally, Cas9 mediates cleavage of target DNA upstream of PAM to create a DSB within the protospacer (FIG. 2A). This example describes an example process for adapting this RNA-programmable nuclease system to direct CRISPR complex activity in the nuclei of eukaryotic cells.

[0154] To improve expression of CRISPR components in mammalian cells, two genes from the SF370 locus 1 of *Streptococcus pyogenes* (*S. pyogenes*) were codon-optimized, Cas9 (SpCas9) and RNase III (SpRNase III). To facilitate nuclear localization, a nuclear localization signal (NLS) was included at the amino (N)- or carboxyl (C)-termini of both SpCas9 and SpRNase III (FIG. 2B). To facilitate visualization of protein expression, a fluorescent protein marker was also included at the N- or C-termini of both proteins (FIG. 2B). A version of SpCas9 with an NLS attached to both N- and C-termini (2xNLS-SpCas9) was also generated. Constructs containing NLS-fused SpCas9 and SpRNase III were transfected into 293FT human embryonic kidney (HEK) cells, and the relative positioning of the NLS to SpCas9 and SpRNase III was found to affect their nuclear localization efficiency.

Whereas the C-terminal NLS was sufficient to target SpR-Nase III to the nucleus, attachment of a single copy of these particular NLS's to either the N- or C-terminus of SpCas9 was unable to achieve adequate nuclear localization in this system. In this example, the C-terminal NLS was that of nucleoplasmin (KRPAATKKAGQAKKKK), and the C-terminal NLS was that of the SV40 large T-antigen (PKKKRKV). Of the versions of SpCas9 tested, only 2xNLS-SpCas9 exhibited nuclear localization (FIG. 2B).

[0155] The tracrRNA from the CRISPR locus of *S. pyogenes* SF370 has two transcriptional start sites, giving rise to two transcripts of 89-nucleotides (nt) and 171nt that are subsequently processed into identical 75nt mature tracrRNAs. The shorter 89nt tracrRNA was selected for expression in mammalian cells (expression constructs illustrated in FIG. 6, with functionality as determined by results of Surveyor assay shown in FIG. 6B). Transcription start sites are marked as +1, and transcription terminator and the sequence probed by northern blot are also indicated. Expression of processed tracrRNA was also confirmed by Northern blot. FIG. 7C shows results of a Northern blot analysis of total RNA extracted from 293FT cells transfected with U6 expression constructs carrying long or short tracrRNA, as well as SpCas9 and DR-EMX1(1)-DR. Left and right panels are from 293FT cells transfected without or with SpRNase III, respectively. U6 indicate loading control blotted with a probe targeting human U6 snRNA. Transfection of the short tracrRNA expression construct led to abundant levels of the processed form of tracrRNA (~75 bp). Very low amounts of long tracrRNA are detected on the Northern blot.

[0156] To promote precise transcriptional initiation, the RNA polymerase III-based U6 promoter was selected to drive the expression of tracrRNA (FIG. 2C). Similarly, a U6 promoter-based construct was developed to express a pre-crRNA array consisting of a single spacer flanked by two direct repeats (DRs, also encompassed by the term "tracr-mate sequences"; FIG. 2C). The initial spacer was designed to target a 33-base-pair (bp) target site (30-bp protospacer plus a 3-bp CRISPR motif (PAM) sequence satisfying the NGG recognition motif of Cas9) in the human EALX1 locus (FIG. 2C), a key gene in the development of the cerebral cortex.

[0157] To test whether heterologous expression of the CRISPR system (SpCas9, SpRNase III, tracrRNA, and pre-crRNA) in mammalian cells can achieve targeted cleavage of mammalian chromosomes, HEK 293FT cells were transfected with combinations of CRISPR components. Since DSBs in mammalian nuclei are partially repaired by the non-homologous end joining (NHEJ) pathway, which leads to the formation of indels, the Surveyor assay was used to detect potential cleavage activity at the target EMX1 locus (see e.g. Guschin et al., 2010, Methods Mol Biol 649: 247). Co-transfection of all four CRISPR components was able to induce up to 5.0% cleavage in the protospacer (see FIG. 2D). Co-transfection of all CRISPR components minus SpRNase III also induced up to 4.7% indel in the protospacer, suggesting that there may be endogenous mammalian RNases that are capable of assisting with crRNA maturation, such as for example the related Dicer and Drosha enzymes. Removing any of the remaining three components abolished the genome cleavage activity of the CRISPR system (FIG. 2D). Sanger sequencing of amplicons containing the target locus verified the cleavage activity: in 43 sequenced clones, 5 mutated alleles (11.6%) were found. Similar experiments using a variety of guide sequences produced indel percentages as high as

29% (see FIGS. 4-8, 10 and 11). These results define a three-component system for efficient CRISPR-mediated genome modification in mammalian cells.

[0158] To optimize the cleavage efficiency, Applicants also tested whether different isoforms of tracrRNA affected the cleavage efficiency and found that, in this example system, only the short (89-bp) transcript form was able to mediate cleavage of the human EM-AV genomic locus. FIG. 9 provides an additional Northern blot analysis of crRNA processing in mammalian cells. FIG. 9A illustrates a schematic showing the expression vector for a single spacer flanked by two direct repeats (DR-EMX1(1)-DR). The 30 bp spacer targeting the human EMX1 locus protospacer 1 and the direct repeat sequences are shown in the sequence beneath FIG. 9A. The line indicates the region whose reverse-complement sequence was used to generate Northern blot probes for EMX1(1) crRNA detection. FIG. 9B shows a Northern blot analysis of total RNA extracted from 293FT cells transfected with U6 expression constructs carrying DR-EMX1(1)-DR. Left and right panels are from 293FT cells transfected without or with SpRNase III respectively. DR-EMX1(1)-DR was processed into mature crRNAs only in the presence of SpCas9 and short tracrRNA and was not dependent on the presence of SpRNase III. The mature crRNA detected from transfected 293FT total RNA is ~33 bp and is shorter than the 39-42 bp mature crRNA from *S. pyogenes*. These results demonstrate that a CRISPR system can be transplanted into eukaryotic cells and reprogrammed to facilitate cleavage of endogenous mammalian target polynucleotides.

[0159] FIG. 2 illustrates the bacterial CRISPR system described in this example. FIG. 2A illustrates a schematic showing the CRISPR locus 1 from *Streptococcus pyogenes* SF370 and a proposed mechanism of CRISPR-mediated DNA cleavage by this system. Mature crRNA processed from the direct repeat-spacer array directs Cas9 to genomic targets consisting of complementary protospacers and a protospacer-adjacent motif (PAM). Upon target-spacer base pairing, Cas9 mediates a double-strand break in the target DNA. FIG. 2B illustrates engineering of *S. pyogenes* Cas9 (SpCas9) and RNase III (SpRNase III) with nuclear localization signals (NLSs) to enable import into the mammalian nucleus. FIG. 2C illustrates mammalian expression of SpCas9 and SpRNase III driven by the constitutive EF1a promoter and tracrRNA and pre-crRNA array (DR-Spacer-DR) driven by the RNA Pol3 promoter U6 to promote precise transcription initiation and termination. A protospacer from the human EMX1 locus with a satisfactory PAM sequence is used as the spacer in the pre-crRNA array. FIG. 2D illustrates surveyor nuclease assay for SpCas9-mediated minor insertions and deletions. SpCas9 was expressed with and without SpRNase III, tracrRNA, and a pre-crRNA array carrying the EMX1-target spacer. FIG. 2E illustrates a schematic representation of base pairing between target locus and EMX1-targeting crRNA, as well as an example chromatogram showing a micro deletion adjacent to the SpCas9 cleavage site. FIG. 2F illustrates mutated alleles identified from sequencing analysis of 43 clonal amplicons showing a variety of micro insertions and deletions. Dashes indicate deleted bases, and non-aligned or mismatched bases indicate insertions or mutations. Scale bar=10 μ m.

[0160] To further simplify the three-component system, a chimeric crRNA-tracrRNA hybrid design was adapted, where a mature crRNA (comprising a guide sequence) is

fused to a partial tracrRNA via a stem-loop to mimic the natural crRNA:tracrRNA duplex (FIG. 3A).

[0161] Guide sequences can be inserted between BbsI sites using annealed oligonucleotides. Protospacers on the sense and anti-sense strands are indicated above and below the DNA sequences, respectively. A modification rate of 6.3% and 0.75% was achieved for the human PVALB and mouse Th loci respectively, demonstrating the broad applicability of the CRISPR system in modifying different loci across multiple organisms. While cleavage was only detected with one out of three spacers for each locus using the chimeric constructs, all target sequences were cleaved with efficiency of indel production reaching 27% when using the co-expressed pre-crRNA arrangement (FIGS. 4 and 5).

[0162] FIG. 5 provides a further illustration that SpCas9 can be reprogrammed to target multiple genomic loci in mammalian cells. FIG. 5A provides a schematic of the human EMX1 locus showing the location of five protospacers, indicated by the underlined sequences. FIG. 5B provides a schematic of the pre-crRNA/tracrRNA complex showing hybridization between the direct repeat region of the pre-crRNA and tracrRNA (top), and a schematic of a chimeric RNA design comprising a 20 bp guide sequence, and tracr mate and tracr sequences consisting of partial direct repeat and tracrRNA sequences hybridized in a hairpin structure (bottom). Results of a Surveyor assay comparing the efficacy of Cas9-mediated cleavage at five protospacers in the human EMX1 locus is illustrated in FIG. 5C. Each protospacer is targeted using either processed pre-crRNA/tracrRNA complex (crRNA) or chimeric RNA (chiRNA).

[0163] Since the secondary structure of RNA can be crucial for intermolecular interactions, a structure prediction algorithm based on minimum free energy and Boltzmann-weighted structure ensemble was used to compare the putative secondary structure of all guide sequences used in our genome targeting experiment (FIG. 3B) (see e.g. Gruber et al., 2008, Nucleic Acids Research, 36: W70). Analysis revealed that in most cases, the effective guide sequences in the chimeric crRNA context were substantially free of secondary structure motifs, whereas the ineffective guide sequences were more likely to form internal secondary structures that could prevent base pairing with the target protospacer DNA. It is thus possible that variability in the spacer secondary structure might impact the efficiency of CRISPR-mediated interference when using a chimeric crRNA.

[0164] FIG. 3 illustrates example expression vectors. FIG. 3A provides a schematic of a bi-cistronic vector for driving the expression of a synthetic crRNA-tracrRNA chimera (chimeric RNA) as well as SpCas9. The chimeric guide RNA contains a 20-bp guide sequence corresponding to the protospacer in the genomic target site. FIG. 3B provides a schematic showing guide sequences targeting the human EMX1, PVALB, and mouse Th loci, as well as their predicted secondary structures. The modification efficiency at each target site is indicated below the RNA secondary structure drawing (EMX1, n=216 amplicon sequencing reads; PVALB, n=224 reads; Th, n=265 reads). The folding algorithm produced an output with each base colored according to its probability of assuming the predicted secondary structure, as indicated by a rainbow scale that is reproduced in FIG. 3B in gray scale. Further vector designs for SpCas9 are shown in FIG. 3A, including single expression vectors incorporating a U6

promoter linked to an insertion site for a guide oligo, and a Cbh promoter linked to SpCas9 coding sequence.

[0165] To test whether spacers containing secondary structures are able to function in prokaryotic cells where CRISPRs naturally operate, transformation interference of protospacer-bearing plasmids were tested in an *E. coli* strain heterologously expressing the *S. pyogenes* SF370 CRISPR locus 1 (FIG. 3C). The CRISPR locus was cloned into a low-copy *E. coli* expression vector and the crRNA array was replaced with a single spacer flanked by a pair of DRs (pCRISPR). *E. coli* strains harboring different pCRISPR plasmids were transformed with challenge plasmids containing the corresponding protospacer and PAM sequences (FIG. 3C). In the bacterial assay, all spacers facilitated efficient CRISPR interference (FIG. 3C). These results suggest that there may be additional factors affecting the efficiency of CRISPR activity in mammalian cells.

[0166] To investigate the specificity of CRISPR-mediated cleavage, the effect of single-nucleotide mutations in the guide sequence on protospacer cleavage in the mammalian genome was analyzed using a series of EMX1-targeting chimeric crRNAs with single point mutations (FIG. 4A). FIG. 4B illustrates results of a Surveyor nuclease assay comparing the cleavage efficiency of Cas9 when paired with different mutant chimeric RNAs. Single-base mismatch up to 12-bp 5' of the PAM substantially abrogated genomic cleavage by SpCas9, whereas spacers with mutations at farther upstream positions retained activity against the original protospacer target (FIG. 4B). In addition to the PAM, SpCas9 has single-base specificity within the last 12-bp of the spacer. Furthermore, CRISPR is able to mediate genomic cleavage as efficiently as a pair of TALE nucleases (TALEN) targeting the same EMX1 protospacer. FIG. 4C provides a schematic showing the design of TALENs targeting E EMX1, and FIG. 4D shows a Surveyor gel comparing the efficiency of TALEN and Cas9 (n=3).

[0167] Having established a set of components for achieving CRISPR-mediated gene editing in mammalian cells through the error-prone NHEJ mechanism, the ability of CRISPR to stimulate homologous recombination (HR), a high fidelity gene repair pathway for making precise edits in the genome, was tested. The wild type SpCas9 is able to mediate site-specific DSBs, which can be repaired through both NHEJ and HR. In addition, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of SpCas9 was engineered to convert the nuclease into a nickase (SpCas9n; illustrated in FIG. 5A) (see e.g. Sapranasaks et al., 2011, Nucleic Acid Research, 39: 9275; Gasiunas et al., 2012, Proc. Natl. Acad. Sci. USA, 109:E2579), such that nicked genomic DNA undergoes the high-fidelity homology-directed repair (HDR). Surveyor assay confirmed that SpCas9n does not generate indels at the EMX1 protospacer target. As illustrated in FIG. 5B, co-expression of EMX1-targeting chimeric crRNA with SpCas9 produced indels in the target site, whereas co-expression with SpCas9n did not (n=3). Moreover, sequencing of 327 amplicons did not detect any indels induced by SpCas9n. The same locus was selected to test CRISPR-mediated HR by co-transfecting HEK 293FT cells with the chimeric RNA targeting EMX1, hSpCas9 or hSpCas9n, as well as a HR template to introduce a pair of restriction sites (HindIII and NheI) near the protospacer. FIG. 5C provides a schematic illustration of the HR strategy, with relative locations of recombination points and primer annealing sequences (arrows). SpCas9 and SpCas9n indeed cata-

lyzed integration of the HR template into the EMX1 locus. PCR amplification of the target region followed by restriction digest with HindIII revealed cleavage products corresponding to expected fragment sizes (arrows in restriction fragment length polymorphism gel analysis shown in FIG. 5D), with SpCas9 and SpCas9n mediating similar levels of HR efficiencies. Applicants further verified HR using Sanger sequencing of genomic amplicons (FIG. 5E). These results demonstrate the utility of CRISPR for facilitating targeted gene insertion in the mammalian genome. Given the 14-bp (12-bp from the spacer and 2-bp from the PAM) target specificity of the wild type SpCas9, the availability of a nickase can significantly reduce the likelihood of off-target modifications, since single strand breaks are not substrates for the error-prone NHEJ pathway.

[0168] Expression constructs mimicking the natural architecture of CRISPR loci with arrayed spacers (FIG. 2A) were constructed to test the possibility of multiplexed sequence targeting. Using a single CRISPR array encoding a pair of EMX1- and PVALB-targeting spacers, efficient cleavage at both loci was detected (FIG. 4F, showing both a schematic design of the crRNA array and a Surveyor blot showing efficient mediation of cleavage). Targeted deletion of larger genomic regions through concurrent DSBs using spacers against two targets within EMX1 spaced by 119 bp was also tested, and a 1.6% deletion efficacy (3 out of 182 amplicons; FIG. 5G) was detected. This demonstrates that the CRISPR system can mediate multiplexed editing within a single genome.

Example 2

CRISPR System Modifications and Alternatives

[0169] The ability to use RNA to program sequence-specific DNA cleavage defines a new class of genome engineering tools for a variety of research and industrial applications. Several aspects of the CRISPR system can be further improved to increase the efficiency and versatility of CRISPR targeting. Optimal Cas9 activity may depend on the availability of free Mg^{2+} at levels higher than that present in the mammalian nucleus (see e.g. Jinek et al., 2012, Science, 337:816), and the preference for an NGG motif immediately downstream of the protospacer restricts the ability to target on average every 12-bp in the human genome. Some of these constraints can be overcome by exploring the diversity of CRISPR loci across the microbial metagenome (see e.g. Makarova et al., 2011, Nat Rev Microbiol, 9:467). Other CRISPR loci may be transplanted into the mammalian cellular milieu by a process similar to that described in Example 1. The modification efficiency at each target site is indicated below the RNA secondary structures. The algorithm generating the structures colors each base according to its probability of assuming the predicted secondary structure. RNA guide spacers 1 and 2 induced 14% and 6.4%, respectively. Statistical analysis of cleavage activity across biological replica at these two protospacer sites is also provided in FIG. 7.

Example 3

Sample Target Sequence Selection Algorithm

[0170] A software program is designed to identify candidate CRISPR target sequences on both strands of an input DNA sequence based on desired guide sequence length and a CRISPR motif sequence (PAM) for a specified CRISPR enzyme. For example, target sites for Cas9 from *S. pyogenes*, with PAM sequences NGG, may be identified by searching for 5'-N_x-NGG-3' both on the input sequence and on the

reverse-complement of the input. Likewise, target sites for Cas9 of *S. thermophilus* CRISPR1, with PAM sequence NNAGAAW, may be identified by searching for 5'-N_x-NNA-GAAW-3' both on the input sequence and on the reverse-complement of the input. Likewise, target sites for Cas9 of *S. thermophilus* CRISPR3, with PAM sequence NGGNG, may be identified by searching for 5'-N_x-NGGNG-3' both on the input sequence and on the reverse-complement of the input. The value "x" in N_x may be fixed by the program or specified by the user, such as 20.

[0171] Since multiple occurrences in the genome of the DNA target site may lead to nonspecific genome editing, after identifying all potential sites, the program filters out sequences based on the number of times they appear in the relevant reference genome. For those CRISPR enzymes for which sequence specificity is determined by a 'seed' sequence, such as the 11-12 bp 5' from the PAM sequence, including the PAM sequence itself, the filtering step may be based on the seed sequence. Thus, to avoid editing at additional genomic loci, results are filtered based on the number of occurrences of the seed:PAM sequence in the relevant genome. The user may be allowed to choose the length of the seed sequence. The user may also be allowed to specify the number of occurrences of the seed:PAM sequence in a genome for purposes of passing the filter. The default is to screen for unique sequences. Filtration level is altered by changing both the length of the seed sequence and the number of occurrences of the sequence in the genome. The program may in addition or alternatively provide the sequence of a guide sequence complementary to the reported target sequence(s) by providing the reverse complement of the identified target sequence(s).

[0172] Further details of methods and algorithms to optimize sequence selection can be found in U.S. application Ser. No. TBA (Broad Reference BI-2012/084 44790.11. 2022); incorporated herein by reference.

Example 4

Evaluation of Multiple Chimeric crRNA-tracrRNA Hybrids

[0173] This example describes results obtained for chimeric RNAs (chiRNAs; comprising a guide sequence, a tracr mate sequence, and a tracr sequence in a single transcript) having tracr sequences that incorporate different lengths of wild-type tracrRNA sequence. FIG. 18a illustrates a schematic of a bicistronic expression vector for chimeric RNA and Cas9. Cas9 is driven by the CBh promoter and the chimeric RNA is driven by a U6 promoter. The chimeric guide RNA consists of a 20 bp guide sequence (Ns) joined to the tracr sequence (running from the first "U" of the lower strand to the end of the transcript), which is truncated at various positions as indicated. The guide and tracr sequences are separated by the tracr-mate sequence GUUUUAGAGCUA followed by the loop sequence GAAA. Results of SURVEYOR assays for Cas9-mediated indels at the human EMX1 and PVALB loci are illustrated in FIGS. 18b and 18c, respectively. Arrows indicate the expected SURVEYOR fragments. ChiRNAs are indicated by their "+n" designation, and crRNA refers to a hybrid RNA where guide and tracr sequences are expressed as separate transcripts. Quantification of these results, performed in triplicate, are illustrated by histogram in FIGS. 11a and 11b, corresponding to FIGS. 10b and 10c, respectively ("N.D." indicates no indels detected). Protospacer IDs and their corresponding genomic target, protospacer sequence, PAM sequence, and strand location are provided in Table D. Guide sequences were designed to be complementary to the entire protospacer sequence in the case of separate transcripts in the hybrid system, or only to the underlined portion in the case of chimeric RNAs.

TABLE D

protospacer	genomic				
ID	target	protospacer sequence (5' to 3')	PAM	Strand	
1	EMX1	GGACATCGATGTCACCTCCAATGACTAG GG	TGG	+	
2	EMX1	CATTGGAGGTGACATCGATGTCCTCCCC AT	TGG	-	
3	EMX1	GGAAGGGCCTGAGTCCGAGCAGAAGAA GAA	GGG	+	
4	PVALB	GGTGGCGAGAGGGGCCGAGATTGGGTGT TC	AGG	+	
5	PVALB	ATGCAGGAGGGTGGCGAGAGGGGCCGA GAT	TGG	+	

Cell Culture and Transfection

[0174] Human embryonic kidney (HEK) cell line 293FT (Life Technologies) was maintained in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (HyClone), 2 mM GlutaMAX (Life Technologies), 100U/mL penicillin, and 100 µg/mL streptomycin at 37° C. with 5% CO₂ incubation. 293FT cells were seeded onto 24-well plates (Corning) 24 hours prior to transfection at a density of 150,000 cells per well. Cells were transfected using Lipofectamine 2000 (Life Technologies) following the manufacturer's recommended protocol. For each well of a 24-well plate, a total of 500 ng plasmid was used.

SURVEYOR Assay for Genome Modification

[0175] 293FT cells were transfected with plasmid DNA as described above. Cells were incubated at 37° C. for 72 hours post-transfection prior to genomic DNA extraction. Genomic DNA was extracted using the QuickExtract DNA Extraction Solution (Epicentre) following the manufacturer's protocol. Briefly, pelleted cells were resuspended in QuickExtract solution and incubated at 65° C. for 15 minutes and 98° C. for 10 minutes. The genomic region flanking the CRISPR target site for each gene was PCR amplified (primers listed in Table E), and products were purified using QiaQuick Spin Column (Qiagen) following the manufacturer's protocol. 400 ng total of the purified PCR products were mixed with 2 µl 10×Taq DNA Polymerase PCR buffer (Enzymatics) and ultrapure water to a final volume of 20 µl, and subjected to a re-annealing process to enable heteroduplex formation: 95° C. for 10 min, 95° C. to 85° C. ramping at -2° C./s, 85° C. to 25° C. at -0.25° C./s, and 25° C. hold for 1 minute. After re-annealing, products were treated with SURVEYOR nuclease and SURVEYOR enhancer S (Transgenomics) following the manufacturer's recommended protocol, and analyzed on 4-20% Novex TBE poly-acrylamide gels (Life Technologies). Gels were stained with SYBR Gold DNA stain (Life Technologies) for 30 minutes and imaged with a Gel Doc gel imaging system (Bio-rad). Quantification was based on relative band intensities.

TABLE E

primer name	genomic target	primer sequence (5' to 3')
Sp-EMX1-F	EMX1	AAAACCACCTTCTCTGTGGC
Sp-EMX1-R	EMX1	GGAGATTGGAGACACGGAGAG
Sp-PVALB-F	PVALB	CTGGAAGCCAATGCCTGAC
Sp-PVALB-R	PVALB	GGCAGCAAACCTCTTGTCCT

Computational Identification of Unique CRISPR Target Sites

[0176] To identify unique target sites for the *S. pyogenes* SF370 Cas9 (SpCas9) enzyme in the human, mouse, rat, zebrafish, fruit fly, and *C. elegans* genome, we developed a software package to scan both strands of a DNA sequence and identify all possible SpCas9 target sites. For this example, each SpCas9 target site was operationally defined as a 20 bp sequence followed by an NGG protospacer adjacent motif (PAM) sequence, and we identified all sequences satisfying this 5'-N₂₀-NGG-3' definition on all chromosomes. To prevent non-specific genome editing, after identifying all potential sites, all target sites were filtered based on the number of times they appear in the relevant reference genome. To take advantage of sequence specificity of Cas9 activity conferred by a 'seed' sequence, which can be, for example, approximately 11-12 bp sequence 5' from the PAM sequence, 5'-NNNNNNNNNN-NGG-3' sequences were selected to be unique in the relevant genome. All genomic sequences were downloaded from the UCSC Genome Browser (Human genome hg19, Mouse genome mm9, Rat genome rn5, Zebrafish genome danRer7, *D. melanogaster* genome dm4 and *C. elegans* genome ce10). The full search results are available to browse using UCSC Genome Browser information. An example visualization of some target sites in the human genome is provided in FIG. 22.

[0177] Initially, three sites within the EMX1 locus in human HEK 293FT cells were targeted. Genome modification efficiency of each chiRNA was assessed using the SURVEYOR nuclease assay, which detects mutations resulting from DNA double-strand breaks (DSBs) and their subsequent repair by the non-homologous end joining (NHEJ) DNA damage repair pathway. Constructs designated chiRNA(+n) indicate that up to the +n nucleotide of wild-type tracrRNA is included in the chimeric RNA construct, with values of 48, 54, 67, and 85 used for n. Chimeric RNAs containing longer

fragments of wild-type tracrRNA (chiRNA(+67) and chiRNA(+85)) mediated DNA cleavage at all three EMX1 target sites, with chiRNA(+85) in particular demonstrating significantly higher levels of DNA cleavage than the corresponding crRNA/tracrRNA hybrids that expressed guide and tracr sequences in separate transcripts (FIGS. 10b and 10a). Two sites in the PVALB locus that yielded no detectable cleavage using the hybrid system (guide sequence and tracr sequence expressed as separate transcripts) were also targeted using chiRNAs. chiRNA(+67) and chiRNA(+85) were able to mediate significant cleavage at the two PVALB protospacers (FIGS. 10c and 10b).

[0178] For all five targets in the EMX1 and PVALB loci, a consistent increase in genome modification efficiency with increasing tracr sequence length was observed. Without wishing to be bound by any theory, the secondary structure formed by the 3' end of the tracrRNA may play a role in enhancing the rate of CRISPR complex formation. An illustration of predicted secondary structures for each of the chimeric RNAs used in this example is provided in FIG. 21. The secondary structure was predicted using RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>) using minimum free energy and partition function algorithm. Pseudocolor for each based (reproduced in grayscale) indicates the probability of pairing. Because chiRNAs with longer tracr sequences were able to cleave targets that were not cleaved by native CRISPR crRNA/tracrRNA hybrids, it is possible that chimeric RNA may be loaded onto Cas9 more efficiently than its native hybrid counterpart. To facilitate the application of Cas9 for site-specific genome editing in eukaryotic cells and organisms, all predicted unique target sites for the *S. pyogenes* Cas9 were computationally identified in the human, mouse, rat, zebra fish, *C. elegans*, and *D. melanogaster* genomes. Chimeric RNAs can be designed for Cas9 enzymes from other microbes to expand the target space of CRISPR RNA-programmable nucleases.

[0179] FIGS. 11 and 21 illustrate exemplary bicistronic expression vectors for expression of chimeric RNA including up to the +85 nucleotide of wild-type tracr RNA sequence, and SpCas9 with nuclear localization sequences. SpCas9 is expressed from a CBh promoter and terminated with the bGH polyA signal (bGH pA). The expanded sequence illustrated immediately below the schematic corresponds to the region surrounding the guide sequence insertion site, and includes, from 5' to 3', portion of the U6 promoter (first shaded region), BbsI cleavage sites (arrows), partial direct repeat (tracr mate sequence GTTTTAGAGCTA, underlined), loop sequence GAAA, and +85 tracr sequence (underlined sequence following loop sequence). An exemplary guide sequence insert is illustrated below the guide sequence insertion site, with nucleotides of the guide sequence for a selected target represented by an "N".

[0180] Sequences described in the above examples are as follows (polynucleotide sequences are 5' to 3'):

[0181] U6-short tracrRNA (*Streptococcus pyogenes* SF370):

```
GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGC
TGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG
TACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAAGTT
TTAAATTTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA
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-continued

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GTATTTTCGATTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCG
GAACCATTCAAAACAGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAGTGGCACCGAGTCGGTGCTTTTTTTT
(bold = tracrRNA sequence; underline = terminator sequence)
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[0182] U6-long tracrRNA (*Streptococcus pyogenes* SF370):

```
GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGC
TGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG
TACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAAGTT
TTAAATTTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA
GTATTTTCGATTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCG
GTAGTATTAAGTATTGTTTTATGGCTGATAAATTTCTTTGAATTTCTCCT
TGATTATTTGTTATAAAAGTTATAAAATAATCTTGTGGAACCATTCAAA
ACAGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGT
GGCACCGAGTCCGTGCTTTTTTT
```

[0183] U6-DR-BbsI backbone-DR (*Streptococcus pyogenes* SF370):

```
GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGC
TGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG
TACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAAGTT
TTAAATTTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA
GTATTTTCGATTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCG
GGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACGGGTCTTCGAGAA
GACGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAAC
```

[0184] U6-chimeric RNA-BbsI backbone (*Streptococcus pyogenes* SF370)

```
GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGC
TGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG
TACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAAGTT
TTAAATTTATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA
GTATTTTCGATTCTTGGCTTTATATATCTTGTGAAAGGACGAAACACCG
GGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAATAAG
GCTAGTCCG
```

[0185] NLS-SpCas9-EGFP:

```
MDYKDHGDYKDHDIYKDDDDKMAPKKRKVGIHGVPAADKKYSIGLDI
GTNSVGWAVITDEYKVPKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLEESFLVEEDK
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KHERHPHIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHM
 IKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAI
 LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
 AKLQLSKDYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
 ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG
 YIDGGASQEEFYKFIKPILEKMDGTEELLVKNLREDLLRKQRTFDNGSIP
 HQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVGLARGNSR
 FAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHK
 SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVT
 KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKDKDFLDNEE
 NEDILEDIVLTLTLFEDREMIERLKYAHLFDDKVMKQLKRRYTGWR
 LSRKLINGIRDKQSGKTILDFLKSDFANRNFQMLIHDDSLTFKEDIQKA
 QVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVI
 EMARENQTTQKGQKNSRERMKRIEELKELGSQILKLEHPVENTQLQNEK
 LYLYYLQNGRDMYVDQELDINRLSDYDVHIVPQSFLKDDSIDNKVLR
 TRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSE
 LDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
 KLVSDFRKDFQFYKVRINNYHHAHDAYLNAVGTALIKKYPKLESEFVY
 GDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEITLANGEIRKR
 PLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEVQTGGFSKESI
 LPKRNSDKLIARKKDWDPKYGGFDSPTVAYSVLVAKVEKGSKKLKSV
 KELLGITIMERSSEFKNPIDFLEAKGYKEVKDLIIKLPKYSLEENGR
 KRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDEQKQLFVE
 QHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKHRRDKPIREQAENI
 IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTALIHQSITGLYETRI
 DLSQLGGDAAAVSKGEELFTGVVPIVLVDGDNVGHKFSVSGEGEGDATY
 GKLTCLKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMP
 EGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGH
 KLEYNNYSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIG
 DGPVLLPDNHYLSTQSALS KDPNEKRDHMLLEFVTAAGITLGMDELYK

[0186] SpCas9-EGFP-NLS:

MDKKYSIGLDIGTNSVGWAVITDEYKVP SKKFKVLGNTDRHSIKKNLIGA
 LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRL
 LEESFLVEEDKKHERHPHIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD
 LRILIYALAHMIKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENP
 INASGVDAKAIL SARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
 NFKSNFDLAEDAKLQLSKDYDDDLNLLAQIGDQYADLFLAAKNLSDAI
 LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI

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FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKNLREDLLR
 KQRTFDNGSIPHQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPY
 YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK
 NLPNEKVLPHKSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVD
 LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKLI
 IKDKDFLDNEENEDILEDIVLTLTLFEDREMIERLKYAHLFDDKVMKQ
 LKRRYTGWRSLSRKLINGIRDKQSGKTILDFLKSDFANRNFQMLIHDD
 SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKV
 MGRHKPENIVIEARENQTTQKGQKNSRERMKRIEELKELGSQILKEHP
 VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVHIVPQSFLKDD
 SIDNKVLRTRSDKNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNL
 TKAERGGLSELDKAGFIKRLVETRQITKHVAQILDSRMNTKYDENDKLI
 REVKVITLKS KLVSDFRKDFQFYKVRINNYHHAHDAYLNAVGTALIKK
 YPKLESEFVYGDYKVYDVRKMIKSEQEI GKATAKYFFYSNIMNFFKTEI
 TLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSPQVNIKKTEV
 QTGGFSKESILPKRNSDKLIARKKDWDPKYGGFDSPTVAYSVLVAKVE
 KGSKKLKSVKELLGITIMERSSEFKNPIDFLEAKGYKEVKDLIIKLPK
 YSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPE
 DNEQKQLFVEQHKHYLDEIIIEQISEFSKRVILADANLDKVL SAYNKHRRDK
 PIREQAENI IHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTALIHQ
 SITGLYETRIDLSQLGGDAAAVSKGEELFTGVVPIVLVDGDNVGHKFSV
 SGEGEGDATY GKLTLKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMK
 QHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGID
 FKEDGNILGHKLEYNNYSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA
 DHYQQNTPIGDGPVLLPDNHYLSTQSALS KDPNEKRDHMLLEFVTAAGI
 TLGMDELYKKRPAATKKAGQAKKKK

[0187] NLS-SpCas9-EGFP-NLS:

MDYKDHDGDYKDHDIDYKDDDDKMAPKKRKVKGIHGVPAADKKYSIGLDI
 GTNSVGWAVITDEYKVP SKKFKVLGNTDRHSIKKNLIGALLFDSGETAEA
 TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLLEESFLVEEDK
 KHERHPHIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHM
 IKFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEENPINASGVDAKAI
 LSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
 AKLQLSKDYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTE
 ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG
 YTDGGASQEEFYKFIKPILEKMDGTEELLVKNLREDLLRKQRTFDNGSIP
 HQIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYVGLARGNSR
 FAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPHK

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SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV
 KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKD KDFLDNEE
 NEDILEDIVLTTLTFEDREMIERLKYAHLFDDKVMKQLKRRRYTGWGR
 LSRKLINGIRDKQSGKTILDFLKSDFANRNFQMLIHDDSLTFKEDIQKA
 QVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVI
 EMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKL
 YLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD
 KNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
 DKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
 LVSDFRKDFQFYKVRINNYYHAHDAYLNAVVGITALIKKYPKLESEEVY
 DYKVYDVRMKIAKSEQEIGKATAKYFFYSNIMNPFKTEITLANGEIRKRP
 LIETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKEVQTTGGFSKESIL
 PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAVKVEGKSKKLKSVK
 ELLGITIMERSSEFKNPIDFLEAKGYKEVKDLI IKLPKYSLEFELNGR
 RMLASAGELQKGNELALPSKYVNFYLAHYEKLKGSPEDEQKQLFVEQ
 HKHYLDEIEQISEFSKRVILADANLDKVL SAYNKHDKPIREQAENIIH
 LFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSTGLYETRID
 LSQGGDAAAVSGEELFTGVVPIVLVDGVDVNGHKFSVSGEGEGDATYG
 KLTLKFICTTGKLPVPWPTLVTTLTYGVCFSRYPDHMKQHDFFKSAMPE
 GYVQERTIFFKDDGNYKTRAIEVKFEGDTLVNRIELKGIDFKEDGNILGHK
 LEYNNYSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGD
 GPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAAGITLGMDELYKKR
 PAATKKAGQAKKKK

[0188] NLS-SpCas9-NLS:

MDYKDHGDYKDHDIDYKDDDDKMAPKKRKVGIHGVPAADKKYSIGLDI
 GTNSVGWAVITDEYKVPKSKFKVLGNTRHSIKKNLIGALLFDSGETAEA
 TRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFHRLSESFVEEDK
 KHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVSDTKADLRILIYLAHAM
 IKFRGHFLIEGDLNPDNSVDKLFIQLVQTYNQLFEEPNINASGVDAKAI
 LSARLSKSRRLLENLIAQLPGKKNGFLGNLIALSLGLTPNFKSNFDLAED
 AKLQLSKDITDDDLNLLAQIGDYADFLAAKNLSDAILLSDILRVNTE
 ITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAG
 YIDGGASQEEFYKFIKPILEKMDGTEELLVKNREDLLRKQRTFDNGSIP
 HQIHLGELHAILRQEDFYPLKDNREKIEKILTRIPYVYVGLARGNSR
 FAWMTRKSEETITPWNFEVVVDKGASAQSFIERMTNFDKNLPNEKVLPKH
 SLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTV
 KQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKIIKD KDFLDNEE
 NEDILEDIVLTTLTFEDREMIERLKYAHLFDDKVMKQLKRRRYTGWGR

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LSRKLINGIRDKQSGKTILDFLKSDFANRNFQMLIHDDSLTFKEDIQKA
 QVSGQGDSLHEHIANLAGSPAIIKKGILQTVKVVDELVKVMGRHKPENIVI
 EMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKL
 YLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSD
 KNRGKSDNVPSEEVVKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSEL
 AGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKS
 SDFRKDFQFYKVRINNYYHAHDAYLNAVVGITALIKKYPKLESEFVYGDY
 KVVYDVRMKIAKSEQEIGKATAKYFFYSNIMNPFKTEITLANGEIRKPLI
 ETNGETGEIVWDKGRDFATVRKVLSPQVNIIVKKEVQTTGGFSKESILPK
 RNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVAVKVEGKSKKLKSVKEL
 LGITIMERSSEFKNPIDFLEAKGYKEVKDLI IKLPKYSLEFELNGRKL
 RMLASAGELQKGNELALPSKYVNFYLAHYEKLKGSPEDEQKQLFVEQ
 KHYLDEIEQISEFSKRVILADANLDKVL SAYNKHDKPIREQAENIIH
 FTLTNLGAAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSTGLYETRID
 SQLGGDKRPAATKKAGQAKKKK

[0189] NLS-mCherry-SpRNase3:

MFLFLSLTSLSSSRTLVSKEEDNMIAIKEFMRFKVHMEGVSNGHEFEI
 EGEGERPYEGTQTAKLKVTGGPLPFAWDILSPQFMYGSKAYVKHPADI
 PDYLKLSFPEGFKWERVMNFDGGVVTVTQDSSLQDGEFIYKVLGRGTNF
 PSDGPVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKT
 TYKAKKPVQLPGAYNVNIKLDITSHNEDYITVEQYERAEGRHSTGGMDEL
 YKGSQLEELLSTSFDIQFNDLTLLLETAFTHTSYANEHRLNLVSHNERLE
 FLGDAVLQLIISEYLFAYPKKTEGDMSKLRSMIVREESLAGFSRFCSD
 AYIKLGKGEKSGGRRRDTILGDLFEAFGLALLDKGIDAVRRLKQVMI
 PQVEKGNFERNVYKTCLEFLQTKGDVAIDYQVISEKGPAAKQFEVSI
 VVNGAVLSKGLGKSKKLAEQDAKNALALQSEV

[0190] SpRNase3-mCherry-NLS:

MKQLEELLSTSFDIQFNDLTLLLETAFTHTSYANEHRLNLVSHNERLEFLG
 DAVLQLIISEYLFAYPKKTEGDMSKLRSMIVREESLAGFSRFCSDAYI
 KLKLGKGEKSGGRRRDTILGDLFEAFGLALLDKGIDAVRRLKQVMIQV
 EKNFERNVYKTCLEFLQTKGDVAIDYQVISEKGPAAKQFEVSI
 GAVLSKGLGKSKKLAEQDAKNALALQSEVSGSVSKGEEDNMIAIKEFMR
 KVHMEGVSNGHEFEIEGEGERPYEGTQTAKLKVTGGPLPFAWDILSPQ
 FMYGSKAYVKHPADIPDYKLSFPEGFKWERVMNFDGGVVTVTQDSSLQ
 DGEFIYKVLGRGTNFPDGPVMQKKTMGWEASSERMYPEDGALKGEIKQ
 LKLDGGHYDAEVKTYKAKKPVQLPGAYNVNIKLDITSHNEDYITVEQY
 ERAEGRHSTGGMDELYKKRPAATKKAGQAKKKK

[0191] NLS-SpCas9n-NLS (the D10A nickase mutation is lowercase):

MDYKDHGDYKDHDIDYKDDDDKMAPKKRKRVGIHGVPAADKKYSIGLaI
GTNSVGWAVITDEYKWSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEAT
RLKRTARRRYTRRNRIICYLQEIFSNEMAKVDDSFHRLSEESFLVEEDKK
HERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRILIYALAHMI
KFRGHFLIEGDLNPDNSDVKLFIQLVQTYNQLFEEINPINSAGVDAKAIL
SARLSKSRRLLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDA
KLQLSKDITYDDDLNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEI
TKAPLSASMIKRYDEHHQDLTLKALVRQQLPEKYKEIFFDQSKNGYAGY
IDGGSQEEFYKFIKPILEKMDGTEELLVKNLREDLLRKQRTFDNGSIPH
QIHLGELHAILRRQEDFYFPLKDNREKIEKILTFRIPYYVGPLARGNSRF
AWMTRKSEETITPWNFEVVDKGASQSFIERMTNFDKNLPNEKVLPHKS
LLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVK
QLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLKKIKDKDFLDNEEN
EDILEDIVLTLTLFEDREMIEERLKYAHLFDDKVMKQLKRRRYTGWGRL
SRKLINGIRDKQSGKTIIDFLKSDGFANRNFQLIHDDSLTFKEDIQKAQ
VSGQDLSLHEHIANLAGSPAIIKKGILQTVKVVDLVKVMGRHKPENIVIE
MARENQTTQKGQKNSRERMKRIEIEGIELGSQILKEHPVENTQLQNEKLY
LYYLQNGRDMYVDQELDINRLSDYVDHIVPQSFLKDDSIDNKVLTTRSDK
NRGKSDNVPSSEVVKKMKNYWRQLLNAKLI TQRKFDNLTKAERGGLSELD
KAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSCL
VSDFRKDFQFYKREINNYHHAHDAYLNAVVGTAIIKKYPKLESEFVYGD
YKVYDVRKMIKSEQIEIGKATAKYFFYSNIMNPFKTEITLANGEIRKRPL
IETNGETGEIVWDKGRDFATVRKVLSPQVNI VKKTEVQTGGFSKESILP
KRNSDKLIARKKDWDPKKGFDSP TVAYSVLVVAKEKGSKKLKSVKE
LLGITIMERSFPEKNPIDFLEAKGYKEVKDLIIKLPKYSLFELENGRKR
MLASAGELQKGNELALPSKYVNFYLYLASHYEKLKSGPEDNEQKQLFVEQH
KHYLDEIIEQISEFSKRVLADANLDKVL SAYNKHDKPIREQAENIIHL
FTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDTLHQSI TGLYETRIDL
SQLGDKRPAATTKAGQAKKKK

[0192] hEMX1-HR Template-HindII-NheI:

GAATGCTGCCCTCAGACCCGCTTCTCCTGTCTGTCTGTCCAAGGAG
AATGAGGTCTCACTGGTGGATTTGCGACTACCTGAGGAGCTGGCACCTG
AGGGACAAGGCCCCACCTGCCAGCTCCAGCCTCTGATGAGGGGTGGG
AGAGAGCTACATGAGGTTGTCTAAGAAAGCCTCCCCTGAAGGAGACCACAC
AGTGTGTGAGGTTGAGTCTCTAGCAGCGGTCTGTGCCCCAGGGATA
GTCTGGCTGTCCAGGCACTGCTCTTGATAAAACACCACCTCCTAGTTATG
AAACCATGCCATTCTGCCTCTCTGTATGGAAGAGCATGGGCTGGCC

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CGTGGGGTGGTGTCCACTTTAGGCCCTGTGGGAGATCATGGGAACCCACG
CAGTGGGTCATAGGCTCTCTCATTTACTACTCACATCCACTCTGTGAAGA
AGCGATTATGATCTCTCCTCTAGAACTCGTAGAGTCCCATGTCTGCCGG
CTTCCAGAGCCTGCACTCCTCCACCTTGCTTGGCTTTGCTGGGGCTAGA
GGAGCTAGGATGCACAGCAGCTCTGTGACCCCTTGTGTTGAGAGGAACAGG
AAAACCACCCCTCTCTCTGGCCCACTGTGTCTCTTCTGCCCCTGCCATC
CCCTTCTGTGAATGTTAGACCCATGGGAGCAGCTGGTCAGAGGGGACCCC
GGCCTGGGGCCCCCTAACCTATGTAGCCTCAGTCTTCCCATCAGGCTCTC
AGCTCAGCCTGAGTGTGAGGCCCCAGTGGCTGCTCTGGGGGCTCTCTGA
GTTTCTCATCTGTGCCCTCCCTCCCTGGCCCAGGTGAAGGTGTGGTTCC
AGAACCAGGAGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCT
GAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCAACCGGTGGCGCAT
TGCCACGAAGCAGGCCAATGGGGAGGACATCGATGTCACTCCAATGACa
agcttgetagcGGTGGGCAACCACAAACCCACGAGGGCAGAGTGTGCTT
GCTGTGGCCAGGCCCCCTGCGTGGGGCCAGCTGGACTCTGGCCACTCCC
TGGCCAGGCTTTGGGGAGGCTGGAGTCATGGCCCCACAGGGCTTAAGC
CCGGGGCCGCCATTGACAGAGGGACAAGCAATGGGCTGGCTGAGGCCGG
GACCACTTGGCCTTCTCTCGGAGAGCCTGCCTGCCTGGGCGGGCCCCGC
CGCCACCCGAGCCTCCAGCTGCTCTCCGTGTCTCCAATCTCCCTTTTGT
TTTGATGCATTTCTGTTTTAATTTATTTTCCAGGCACCACTGTAGTTTAG
TGATCCCCAGTGTCCCCCTTCCCTATGGGAATAATAAAAGTCTCTCTCTT
AATGACACGGGCATCCAGCTCCAGCCCCAGAGCCTGGGGTGGTAGATTCC
GGCTCTGAGGGCCAGTGGGGGCTGGTAGAGCAACGCGTTCAGGGCCTGG
GAGCCTGGGGTGGGGTACTGGTGGAGGGGTCAAGGTAATTCATTAAC
CCTCTCTTTTGTGGGGGACCCTGGTCTCTACCTCCAGCTCCACAGCAGG
AGAAACAGGCTAGACATAGGGAAGGGCCATCCTGTATCTTGAGGGAGGAC
AGGCCCAGGTCTTTCTTAACGTATTGAGAGGTGGGAATCAGGCCAGGTA
GTTCAATGGGAGAGGAGAGTGTCTCCCTCTGCCTAGAGACTCTGGTGGC
TTCTCCAGTTGAGGAGAAACCAGAGGAAAGGGGAGGATTGGGGTCTGGGG
GAGGGAACACCATTACAAAGGCTGACGGTTCAGTCCGAAGTCGTGGGC
CCACCAGGATGCTCACCTGTCTTGGAGAACCCTGGGAGGTTGAGACT
GCAGAGACAGGGCTTAAGGCTGAGCCTGCAACAGTCCCCAGTGACTCAG
GGCCTCCTCAGCCCAGAAAGAGCAACGTGCCAGGGCCCCGCTGAGCTCTT
GTGTTCACTG

[0193] NLS-StCsn1-NLS:

MKRPAATKKAGQAKKKKSDLVLGLDIGISVGVGILNKVTGEI IHKNSRI
FPAAQAENNLVRRTNRQGRRLARRKKHRRVRLNRLFEESGLITDFTKISI
NLNPYQLRVKGLTDELSNEELFIALKNMVKHRI SYLDDASDDGNSSVGD

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YAQIVKENSQLETKTPGQIQLERYQTYGQLRGDFTVEKDGGKHRLINVF
 PTSAYRSEALRILQTQQEFNPQITDEFINRYLEILTGRKYYHGPNGNEKS
 RTDYGRYRTSGETLDNIFGILIGKCTFYPDEFRAAKASYTAQEFNLLNDL
 NNLTVPETETKKLSKEQKNQIINYVKNKAMGPAKLFKYIAKLLSCDVADI
 KGYRIDKSGKAEIHTFEAYRKMKTLETLDIEQMDRETLDKLAYVLTNTE
 REGIQEALHEFADGSGFSQKQVDELVQFRKANSSIFGKGWHNFSVKLMME
 LIPELYETSEEQMTILRLGKQKTSSSNKTKYIDEKLLTEEIYNPVVAK
 SVRQAIKIVNAAIKEYGDFDNIVIAMARETNEDDEKKAIQIKQKANKDEK
 DAAMLKAANQYNGKAELPHSVFHHGKQLATKIRLWHQOGERCLYTGTIS
 IHDLINNSNQFEVDHILPLSITPDDSLANKVLVYATANQEKQRTPTQAL
 DSMDDAWSFRELKAFVRESKTLNKKKEYLLTEEDISKFDVRKKFIERNL
 VDTRYASRVVLNALQEHFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDT
 YHHHAVDALIIAASSQLNLWKKQKNTLVSYSEDQLLDIETGELISDDEYK
 ESVPKAPYQHFDVTLKSKEFEDSILFSYQVDSKPNRKISDIATYATRQAK
 VGKDKADETYVLGKIKDIYTQDGYDAFMKIYKKDKSKFLMYRHPQTFEK
 VIEPILENYPNKKINEKGKEVPCNPFLKYKEEHGYIRKYSKKGNGPEIKS
 LKYYDSKLGNHIDITPKDSNNKVVLQSVSPWRADVYFNKTTGKYEILGLK
 YADLQFEKGTGTYKISQEKYNDIKKKEGVDSDSEFKFTLYKNDLLLVKDT
 ETKEQQLFRFLSRTMPKQKHVELKPYDKQKFEGGEALIKVLGNVANSQG
 CKKGLGKSNISIIYKVRTDVLGNQHI IKNEGDKPKLDFKRPAATKKAGQAK
 KKK

[0194] U6-St_tracrRNA(7-97):

GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGC
 TGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACACAAGATATTAG
 TACAAAATACGTGACGTAGAAAGTAATAATTTCTGGGTAGTTTGCAAGTT
 TTAAAATTATGTTTTAAATGGACTATCATATGCTTACCGTAACCTGAAA
 GTATTTGCATTTCTTGCTTTATATATCTTGTGGAAAGGACGAAACACCG
 TTACTTAAATCTTGCAGAAGCTACAAAGATAAGGCTTCATGCCGAAATCA
 ACACCTGTCAATTTATGGCAGGGTGTTCGTTATTTAA

[0195] U6-DR-spacer-DR (*S. pyogenes* SF370)

gagggcctatttcccatgattccttcataatttgcatatacgatacaaggc
 tgtagagagataaattggaattaatttgactgtaaacacaaagatatta
 gtacaaaatacgtgacgtagaaagtaataatttcttggttagtttgagtt
 tttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaa
 agtatttcgatttcttggtttatataatcttggtgaaaggacgaaacacc

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gggttttagagctatgctgttttgaatggtcccaaacNNNNNNNNNNNN
 NNNNNNNNNNNNNNNgtttttagagctatgctgttttgaatggtccca
 aaac**TTTTTTT** (lowercase underline = direct

repeat; N = guide sequence; bold = terminator)

[0196] Chimeric RNA containing+48 tracr RNA (*S. pyo-*
genes SF370)

gagggcctatttcccatgattccttcataatttgcatatacgatacaaggc
 tgtagagagataaattggaattaatttgactgtaaacacaaagatattag
 taaaaatacgtgacgtagaaagtaataatttcttggttagtttgagtt
 ttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaa
 gtatttcgatttcttggtttatataatcttggtgaaaggacgaaacaccN
 NNNNNNNNNNNNNNNgttttgaggctagaaatagcaagttaaaata

aggctagtcagg**TTTTTTT** (N = guide sequence;

first underline = tracr matesequence; second

underline = tracr sequence; bold = terminator)

[0197] Chimeric RNA containing+54 tracr RNA (*S. pyo-*
genes SF370)

gagggcctatttcccatgattccttcataatttgcatatacgatacaaggc
 tgtagagagataaattggaattaatttgactgtaaacacaaagatattag
 taaaaatacgtgacgtagaaagtaataatttcttggttagtttgagtt
 ttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaa
 gtatttcgatttcttggtttatataatcttggtgaaaggacgaaacaccN
 NNNNNNNNNNNNNNNgtttttagagctagaaatagcaagttaaaata

aggctagtcaggttatca**TTTTTTT** (N = guide sequence;

first underline = tracr mate sequence; second

underline = tracr sequence; bold = terminator)

[0198] Chimeric RNA containing+67 tracr RNA (*S. pyo-*
genes SF370)

gagggcctatttcccatgattccttcataatttgcatatacgatacaaggc
 tgtagagagataaattggaattaatttgactgtaaacacaaagatattag
 taaaaatacgtgacgtagaaagtaataatttcttggttagtttgagtt
 ttaaaattatgttttaaaatggactatcatatgcttaccgtaacttgaaa
 gtatttcgatttcttggtttatataatcttggtgaaaggacgaaacaccN

[0199] Chimeric RNA containing+85 tracr RNA (*S. pyogenes* SF370)

[0200] CBh-NLS-SpCas9-NLS

CGTTACATAACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCC
CCCGCCCATTTAGCGTCAATAATGACGTATGTTCCCATAGTAACGCCAATA
GGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCA
CTTGCGAGTACATCAAGTGTATCATATGCCAAGTACGCCCTTATTGACG
TAATGACGGTAAATGGCCCGCTGGCATTATGCCCAGTACATGACCTTAT
GGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
ATGGTCGAGGTTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCC
CTCCCCACCCCAATTTTGTATTATTATTATTTTAAATTATTTTGTGCAG
CGATGGGGGCGGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGG
GGCGAGGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCA
GAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGGCGG
GGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCTGCGACGCTG
CCTTCGCCCGCTGCCCCGCTCCGCCGCGCGCTCGCGCGCCCGCCCCGGC
TCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGGGAGCGGCCCTTCT
CCTCCGGGCTGTAATTAGTGTAGCAAGAGGTAAGGGTTTAAGGGATGGTT
GGTTGGTGGGGTATTAATGTTTAATTACCTGGAGCACCTGCCTGAAATCA
CTTTTTTTCAGGTTGGaccggtgccaccATGGACTATAAGGACCACGACG
GAGACTACAAGGATCATGATATTGATTACAAGACGATGACGATAAGATG
GCCCCAAAGAAGAAGCGGAAGGTGCGGTATCCACGAGTCCCAGCAGCCGG

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CAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGTGGG
 CCGTGATCACCGACGAGTACAAGGTGCCAGCAAGAAATTC AAGTGTCTG
 GGCAACACCGACCGGCACAGCATCAAGAAGAACTGTATCGGAGCCCTGCT
 GTTCGACAGCGCGCAAAACAGCCGAGGCCACCCGGCTGAAGAGAACCGCA
 GAAGAAGATACACCAGACGGAAGAACC GGATCTGCTATCTGCAAGAGATC
 TTCAGCAACGAGATGGCC AAGGTGGACGACAGCTTCTTCCACAGACTGGA
 AGAGTCTCTCTGTGTGAAGAGGATAAGAAGCACGAGCGGCACCCCATCT
 TCGGCAACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACTCT
 TACCACCTGAGAAAGAACTGGTGGACAGCACCGACAAGGCCGACCTGCG
 GCTGATCTATCTGGCCCTGGCCCATGATGATCAAGTTCGGGGGCCACTTCC
 TGATCGAGGGCGACCTGAAACCCGACAACAGCGACGTGGACAAGCTGTTT
 ATCCAGCTGGTGCAGACCTACAACCAGCTGTTTCGAGGAAAACCCCATCAA
 CGCCAGCGGCTGGAGCGCAAGGCCATCCTGTCTGCCAGACTGAGCAAGA
 GCAGACGGCTGGAAAAATCTGATCGCCAGCTGCCCGGCGAGAAGAAGAA
 GGCTGTTTCGGCAACCTGATTGCCCTGAGCCTGGGCCTGACCCCCAATT
 CAAGAGCAACTTCGACCTGGCCGAGGATGCCAACTGCAGCTGAGCAAGG
 ACACCTACGACGACGACCTGGACAACCTGCTGGCCAGATCGGCGACCA
 TACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATCCTGCT
 GAGCGACATCTTGAGAGTGAACACCGAGATCACAAGGCCCCCTTGAGCG
 CCTCTATGATCAAGAGATACGACGAGCACCAACAGGACCTGACCTGTCTG
 AAAGCTCTCGTGC GGCGAGCTGCCTGAGAAGTACAAGAGATTTTCTT
 CGACCAGAGCAAGAACCGGCTACGCCGGCTACATTGACGGCGGAGCCAGCC
 AGGAAGAGTTCTACAAGTTCATCAAGCCCATCTGGAAAAGATGGACGGC
 ACCGAGGAAGTCTCTGTGAAGCTGAACAGAGAGGACCTGCTGCGGAAGCA
 GCGGACCTTCGACAACGGCAGCATCCCCACCAAGATCCACCTGGGAGAGC
 TGCACGCCATTCTGCGCGGGCAGGAAGATTTTTACCCATTCTGAAGGAC
 AACC GGGAAAAGATCGAGAAGATCCTGACCTTCGCGATCCCTACTACGT
 GGGCCCTCTGCCAGGGGAAAACAGCAGATTGCCTGGATGACCAGAAAAG
 GCGAGGAAACCATACCCCCTGGAACCTCGAGGAAGTGGTGGACAAGGGC
 GCTTCCGCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAGAACCT
 GCCCAACGAGAAGGTGTGCCCCAAGCACAGCCTGCTGTACAGTACTTCA
 CCGTGATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGA
 AAGCCCGCTTCTCTGAGCGGCAGCAGAAAAAGGCCATCGTGGACCTGCT
 GTTCAAGACCAACCGGAAAAGTGACCGTGAAGCAGCTGAAAGAGGACTACT
 TCAAGAAAATCGATGCTTCGACTCCGTGGAATCTCCGCGTGGGAAGAT
 CGGTTCACGCCTCCCTGGGCACATACCACGATCTGTGAAAAATTATCAA
 GGACAAGGACTTCTCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATA
 TCGTGCTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGG
 CTGAAAACCTATGCCACCTGTTTCGACGACAAAGTGATGAAGCAGCTGAC

CAGAGAGCAGGCCGAGAATATCATCCACTGTTTACCCTGACCAATCTGG
GAGCCCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGG
TACACCAGCACCAAGAGGTGCTGGACGCCACCTGTATCCACGAGCAT
CACCGGCTGTACGAGACACGGATCGACCTGTCTCAGCTGGGAGGCGACT
TTCTTTTCTTAGCTTGACCAGCTTTCTTAGTAGCAGCAGGACGCTTTAA
 (underline = NLS-hSpCas9-NLS)

NNNNNNNNNNNNNNNNNNNNNgtttttgtactctcaagatttaGAAAtaa
tcttgcagaagctacaaagataaggcttcatgcccqaatcaacaccctgt
catttttatggcagggtgttttcggtatttta**TTTTTT** (N =
 guide sequence; first underline = tracr mate
 sequence; second underline = tracr sequence;
 bold = terminator)

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NNNNNNNNNNNNNNNNNNNNNNNgttttctgactctcaGAAAtgcagaagcta  

caaagataaaggcttcattgcccgaaatcaacaccctgtcattttatggcagg  

gtgtttttcgttatttaaTTTTTT (N = guide sequence;  

first underline = tracrRNA sequence; second  

underline = tracrRNA sequence; bold =  

terminator)
```

NNNNNNNNNNNNNNNNNNNNNNNgttttgtactctcaGAAAtgcagaagcta
caaagataaggctctcatgccgaatcaacaccctgtcattttatggcagg
gtgtTTTTT (N = guide sequence; first underline =
 tracr mate sequence; second underline = tracr
 sequence; bold = terminator)

NNNNNNNNNNNNNNNNNNNNNNNgttattgtactctcaagatttaGAAAtaaa
tcttgcagaagctacaaagataaggcttcatgccgaaatcacacacctgt
catttttatggcagggtgttttcqttatttta**TTTTTT** (N =
guide sequence; first underline = tracr mate
sequence; second underline = tracr sequence;
bold = terminator)

[0205] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR1 Cas9 (with PAM of NNAGAAW)

NNNNNNNNNNNNNNNNNNNNgttattgtactctcaGAAAtgcagaagcta
 caaagataaggcttcatgccgaaatcaacaccctgtcattttatggcagg
 gtgttttcgttatttaa**TTTTTT** (N = guide sequence;
 first underline = tracr mate sequence; second
 underline = tracr sequence; bold = terminator)

[0206] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR1 Cas9 (with PAM of NNAGAAW)

NNNNNNNNNNNNNNNNNNNNgttattgtactctcaGAAAtgcagaagcta
 caaagataaggcttcatgccgaaatcaacaccctgtcattttatggcagg
 gtgt**TTTTTT** (N = guide sequence; first underline =
 tracr mate sequence; second underline = tracr
 sequence; bold = terminator)

[0207] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR1 Cas9 (with PAM of NNAGAAW)

NNNNNNNNNNNNNNNNNNNNgttattgtactctcaagatttaGAAAtaaa
 tcttgcagaagctacaatgataaggcttcatgccgaaatcaacaccctgt
 cattttatggcagggtgttttcgttatttaa**TTTTTT** (N = guide
 sequence; first underline = tracr mate sequence;
 second underline = tracr sequence; bold =
 terminator)

[0208] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR1 Cas9 (with PAM of NNAGAAW)

NNNNNNNNNNNNNNNNNNNNgttattgtactctcaGAAAtgcagaagcta
 caatgataaggcttcatgccgaaatcaacaccctgtcattttatggcagg
 gtgttttcgttatttaa**TTTTTT** (N = guide sequence;
 first underline = tracr mate sequence; second
 underline = tracr sequence; bold = terminator)

[0209] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR1 Cas9 (with PAM of NNAGAAW)

NNNNNNNNNNNNNNNNNNNNgttattgtactctcaGAAAtgcagaagcta
 caatgataaggcttcatgccgaaatcaacaccctgtcattttatggcagg
 gtgt**TTTTTT** (N = guide sequence; first
 underline = tracr mate sequence; second
 underline = tracr sequence; bold = terminator)

[0210] Example chimeric RNA for *S. thermophilus* LMD-9 CRISPR3 Cas9 (with PAM of NGGNG)

NNNNNNNNNNNNNNNNNNNNgttttagagctgtgGAAAcacagcgagtta
 aaataaggcttagtccgtactcaacttgaaaaggtggcaccgattcggt
 gt**TTTTTT** (N = guide sequence; first
 underline = tracr mate sequence; second
 underline = tracr sequence; bold = terminator)

[0211] Codon-optimized version of Cas9 from *S. thermophilus* LMD-9 CRISPR3 locus (with an NLS at both 5' and 3' ends)

ATGAAAAGGCCGGCGCCACGAAAAGGCCGGCCAGGCAAAAAGAAAAA
 GACCAAGCCCTACAGCATCGGCTTGGACATCGGCACCAATAGCGTGGGCT
 GGGCCGTGACCACCGACAACACTACAAGGTGCCAGCAAGAAATGAAGGTG
 CTGGGCAACACCTCCAAGAAGTACATCAAGAAAAACCTGCTGGGCGTGCT
 GCTGTTTCAGACGCGCATTTACAGCCGAGGCGAGACGGCTGAAGAGAACCG
 CCAGACGGCGGTACACCCGGCGGAGAAACAGAATCCTGTATCTGCAAGAG
 ATCTTCAGACCGAGATGGCTACCCTGGACGACGCTTCTTCAGCGGCT
 GGACGACAGCTTCTGTTGCCGACGACAAGCGGGACAGCAAGTACCCCA
 TCTTCGGCAACCTGGTGGAAGAGAAGGCCTACCACGACGAGTTCCCCACC
 ATCTACCACCTGAGAAAGTACCTGGCCGACAGCACAAGAAGGCCGACCT
 GAGACTGGTGTATCTGGCCCTGGCCACATGATCAAGTACCGGGGCCACT
 TCCTGATCGAGGGCGAGTTCAACAGCAAGAACAACGACATCCAGAAGAAC
 TTCCAGGACTTCTTGGACACCTACAACGCCATCTTCGAGAGCGACCTGTCT
 CCTGGAAAACAGCAAGCAGCTGGAAGAGATCGTGAAGGACAAGATCAGCA
 AGCTGGAAAAGAAGGACCGCATCCTGAAGCTGTTCCCCGGCGAGAAGAAC
 AGCGGAATCTTCAGCGAGTTTCTGAAGCTGATCGTGGGCAACAGGCCGA
 CTTTCAGAAAGTCTTCAACCTGGACGAGAAAGCCAGCTGCACCTCAGCA
 AAGAGAGCTACGACGAGGACCTGGAAACCTGCTGGGATATATCGGCGAC
 GACTACAGCGACGTGTTCTGAAGGCCAAGAAGCTGTACGACGTATCCT
 GCTGAGCGGCTTCTGACCGTGACCGACAACGAGACAGAGGCCCCACTGA
 GCAGCGCCATGATTAAGCGGTACAACGAGCACAAAGAGGATCTGGCTCTG
 CTGAAAGAGTACATCCGGAACATCAGCTGAAACCTACAATGAGGTGTT
 CAAGGACGACACCAAGAACGGCTACGCCGGCTACATCGACGGCAAGACCA
 ACCAGGAAGATTTCTATGTGTACCTGAAGAAGCTGCTGGCCGAGTTCGAG
 GGGGCCGACTACTTCTGGAAAAAATCGACCGCGAGGATTTCTGCGGAA
 GCAGCGGACCTTCGACAACGGCAGCATCCCCCTACCAGATCCATCTGCAGG
 AAATGCGGGCCATCCTGGACAAGCAGGCCAAGTTCTACCCATTCTTGGCC
 AAGAACAAGAGCGGATCGAGAAGATCTGACCTTCGCGATCCCTACTAC
 GTGGGCCCCCTGGCCAGAGGCAACAGCGATTTTGCCTGGTCCATCCGGAA

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GCGCAATGAGAAGATCACCCCTGGAACTTCGAGGACGTGATCGACAAAG
 AGTCAGCGCCGAGGCCTTCAATCAACCGGATGACCAGCTTCGACCTGTAC
 CTGCCCAGGAAAAGGTGCTGCCAAGCACAGCCTGCTGTACGAGACATT
 CAATGTGTATAACGAGCTGACCAAAGTGCAGTTTATCGCCGAGTCTATGC
 GGGACTACCACTTCTGGACTCCAAGCAGAAAAAGGACATCGTCGGCTG
 TACTTCAAGGACAAGCGGAAAGTGACCGATAAGGACATCATCGAGTACCT
 GCAGCCATCTACGGCTACGATGGCATCGAGCTGAAGGGCATCGAGAAGC
 AGTTCAACTCCAGCTGAGCACATACACGACCTGCTGAACATTATCAAC
 GACAAAGAATTTCTGACGACTCCAGCAACGAGGCCATCATCGAAGAGAT
 CATCCACACCCTGACCATCTTTGAGGACCGCGAGATGATCAAGCAGCGGC
 TGAGCAAGTTCGAGAACATCTTCGACAAGAGCGTGTGAAAAGCTGAGC
 AGACGGCACTACACCGCTGGGGCAAGCTGAGCGCCAAGCTGATCAACGG
 CATCCGGGACGAGAAGTCCGGCAACACAATCCTGGACTACCTGATCGACG
 ACGGCATCAGCAACCGGAACCTTATGCAGCTGATCCACGACGACGCCCTG
 AGCTTCAAGAAGAAGATCCAGAAGGCCAGATCATCGGGGACGAGGACAA
 GGGCAACATCAAAGAAGTCGTGAAGTCCCTGCCCGGCGAGCCCCGCCATCA
 AGAAGGAATCCTGCAGAGCATCAAGATCGTGGACGAGCTCGTGAAAGTG
 ATGGGCGGCAGAAAGCCGAGAGCATCGTGGTGGAAATGGCTAGAGAGAA
 CCAGTACACCAATCAGGGCAAGAGCAACAGCCAGCAGAGACTGAAGAGAC
 TGGAAAAGTCCCTGAAAGAGCTGGGCAGCAAGATTCTGAAAGAGAATATC
 CCTGCCAAGCTGTCCAAGATCGACAACAACGCCCTGCAGAACGACCGGCT
 GTACCTGTACTACCTGCAGAAATGGCAAGGACATGTATACAGGCGACGACC
 TGGATATCGACCGCTGAGCAACTACGACATCGACCATATTATCCCCAG
 GCCTTCTGAAAGACAACAGCATTGACAACAAAGTGTGGTGTCTCCGC
 CAGCAACCGCGGCAAGTCCGATGATGTGCCAGCCTGGAAGTCGTGAAA
 AGAGAAGACCTTCTGGTATCAGCTGTGAAAAGCAAGCTGATTAGCCAG
 AGGAAGTTCGACAACCTGACCAAGGCCGAGAGAGGCGCCTGAGCCCTGA
 AGATAAGCCGGCTTCATCCAGAGACAGCTGGTGGAAACCCGGCAGATCA
 CCAAGCACGTGGCCAGACTGCTGGATGAGAAGTTTAACAACAAGAAGGAC
 GAGAACAACCGGCGGTGCGGACCGTGAAGATCATCACCTGAAGTCCAC
 CCTGGTGTCCAGTTCGGGAAGGACTTCGAGCTGTATAAAGTGCGCGAGA
 TCAATGACTTTCACACGCCCCACGACGCTACCTGAATGCCGTGGTGGCT
 TCCGCCCTGTGAGAAGTACCTAAGCTGGAACCCGAGTTCGTGTACGG
 CGACTACCCCAAGTACAATCCTTCAGAGAGCGGAAGTCCGCCACCGAGA
 AGGTGTACTTCTACTCCAACATCATGAATATCTTTAAGAAGTCCATCTCC
 CTGGCCGATGGCAGAGTATCGAGCGGCCCTGATCGAAGTGAACGAAGA
 GACAGGCGAGAGCGTGTGGAAACAAAGAAAGCGACCTGGCCACCGTGGGC
 GGGTGTGAGTTATCCTCAAGTGAATGTCTGAAGAAGGTGGAAGAACAG
 AACCACGGCCTGGATCGGGGCAAGCCCAAGGGCTGTTCAACGCCAACCT

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GTCCAGCAAGCCTAAGCCCACTCCAACGAGAATCTCGTGGGGGCCAAAG
 AGTACCTGGACCCTAAGAAGTACGGCGGATACGCCGGCATCTCCAATAGC
 TTCACCGTGCTCGTGAAGGGCACAATCGAGAAGGGCGCTAAGAAAAAGAT
 CACAAACGTGCTGGAATTTAGGGGATCTCTATCTGGACCGGATCAACT
 ACCGGAAGGATAAGCTGAACCTTCTGCTGAAAAAGGCTACAAGGACATT
 GAGCTGATTATCGAGCTGCCTAAGTACTCCCTGTTGGAAGTGAAGCGACGG
 CTCCAGACGGATGCTGGCCTCCATCCTGTCCACCAACAACAAGCGGGGCG
 AGATCCACAAGGGAACAGATCTTCTGAGCCAGAAATTTGTGAAAGTGA
 CTGTACCACGCCAAGCGGATCTCCAACACCATCAATGAGAACCACCGGAA
 ATACGTGGAAAACCAAGAAAGAGTTTGAAGAACTGTTCTACTACATCC
 TGGAGTTCAACGAGAACTATGTGGGAGCCAAGAAGAACGGCAAACTGCTG
 AACTCCGCCTTCCAGAGCTGGCAGAACACAGCATCGACGAGCTGTGCAG
 CTCTTCATCGGCCCTACCGGCGAGCGAGCGGAAGGGACTGTTTGAAGTGA
 CCTCCAGAGGCTCTGCCGCGACTTTGAGTTCCTGGGAGTGAAGATCCCC
 CGGTACAGAGACTACACCCCTCTAGTCTGCTGAAGGACGCCACCCTGAT
 CCACCAGAGCGTGACCGGCTGTACGAAACCCGGATCGACCTGGCTAAGC
 TGGGCGAGGGAAGCGTCTGTGTCTACTAAGAAAGCTGGTCAAGCTAAG
 AAAAAAGAAATAA

Example 5

Optimization of the Guide RNA for *Streptococcus pyogenes* Cas9 (Referred to as SpCas9)

[0212] Applicants mutated the tracrRNA and direct repeat sequences, or mutated the chimeric guide RNA to enhance the RNAs in cells.

[0213] The optimization is based on the observation that there were stretches of thymines (Ts) in the tracrRNA and guide RNA, which might lead to early transcription termination by the pol 3 promoter. Therefore Applicants generated the following optimized sequences. Optimized tracrRNA and corresponding optimized direct repeat are presented in pairs.

[0214] Optimized tracrRNA 1 (mutation underlined):

GGAACCATTCATAACAGCATAGCAAGTTATAAATAAGGCTAGTCCGTTAT
 CAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTT

[0215] Optimized direct repeat 1 (mutation underlined):

GTTaTAGAGCTATGCTGTTaTGAATGGTCCCAAAAC

[0216] Optimized tracrRNA 2 (mutation underlined):

GGAACCATTCAATAACAGCATAGCAAGTTAATAAATAAGGCTAGTCCGTTAT
 CAACTTGAAAAAGTGGCACCGAGTCGGTGTCTTTT

[0217] Optimized direct repeat 2 (mutation underlined):

GTaTTAGAGCTATGCTGTaTTGAATGGTCCCAAAAC

[0218] Applicants also optimized the chimeric guideRNA for optimal activity in eukaryotic cells.

[0219] Original guide RNA:

NNNNNNNNNNNNNNNNNNNGTTTATAGAGCTAGAAATAGCAAGTTAAAA
TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTT
TTTTT

[0220] Optimized chimeric guide RNA sequence 1:

NNNNNNNNNNNNNNNNNNNGTaTTAGAGCTAGAAATAGCAAGTTaTA
TAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTT
TTTTT

Optimized chimeric guide RNA sequence 2:

NNNNNNNNNNNNNNNNNNNGTTTATAGAGCTATGCTGTTTTGGAAACAAA
ACAGCATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAG
TGGCACCGAGTCGGTGCTTTTTTTT

[0221] Optimized chimeric guide RNA sequence 3:

NNNNNNNNNNNNNNNNNNNGTaTTAGAGCTATGCTGTaTTGGAAACAA
TACAGCATAGCAAGTTaTAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGCACCGAGTCGGTGCTTTTTTTT

[0222] Applicants showed that optimized chimeric guide RNA works better as indicated in FIG. 9. The experiment was conducted by co-transfecting 293FT cells with Cas9 and a U6-guide RNA DNA cassette to express one of the four RNA forms shown above. The target of the guide RNA is the same target site in the human Emx 1 locus: "GTCACCTCCAAT-GACTAGGG"

Example 6

Optimization of *Streptococcus thermophilus* LMD-9 CRISPR1 Cas9 (referred to as St1Cas9)

[0223] Applicants designed guide chimeric RNAs as shown in FIG. 12.

[0224] The St1Cas9 guide RNAs can under go the same type of optimization as for SpCas9 guide RNAs, by breaking the stretches of poly thymines (Ts).

Example 7

Improvement of the Cas9 System for In Vivo Application

[0225] Applicants conducted a Metagenomic search for a Cas9 with small molecular weight. Most Cas9 homologs are fairly large. For example the SpCas9 is around 1368aa long, which is too large to be easily packaged into viral vectors for delivery. Some of the sequences may have been mis-anno-

tated and therefore the exact frequency for each length may not necessarily be accurate. Nevertheless it provides a glimpse at distribution of Cas9 proteins and suggest that there are shorter Cas9 homologs.

[0226] Through computational analysis, Applicants found that in the bacterial strain *Campylobacter*, there are two Cas9 proteins with less than 1000 amino acids. The sequence for one Cas9 from *Campylobacter jejuni* is presented below. At this length, CjCas9 can be easily packaged into AAV, lentiviruses, Adenoviruses, and other viral vectors for robust delivery into primary cells and in vivo in animal models.

[0227] >*Campylobacter jejuni* Cas9 (CjCas9)

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRL
ARSARKRLARRKARLNHLKHLIANEFKLNIEDYQSFDESLAKAYKGLIS
PYELRFRALNELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIK
QNEEKLANYSQSVGEYLYKEYFQKFKENSKFTNVRNKESYERCIASQFL
KDELKLIFFKQREFGFSPSKKFEEVLSVAFYKRALKDFSHLVGNCSFFT
DEKRAPKNSPLAFMFVALTRIINLLNLIKNTGILYTKDDLNALLNEVLK
NGTLTYKQTKLLGLSDDYEFKGEKGTIFYIEFKKYKEFIKALGEHNSQD
DLNEIAKLDITLIKDEIKLKALAKYDLNQNQIDLSKLEFKDHLNISFK
ALKLVTPMLLEGKKYDEACNELNLKVAINEDKDKFLPAFNETYYKDEVTN
PVVLRRAIKYRKVLNALLKKYGVHKKINIELAREVGKNHSQRAKIEKEQN
ENYKAKKDAELECEKLGKINSKNIKLRFLKEQKEFCAYSSEKIKISDL
QDEKMLEIDHIYPYRSFDDSYMKNVLVPTKQNEKELNQTFEAFGNDAS
KWQKIEVLAKNLPKKQKRILDKNYKDKQKNFDRNLNDTRYIARLVNLN
TKDYLDPLPLSDDENTKLNQKQSKVHVEAKSGMLTSALRHTWGFSKAD
RNNHLHHAIDAVIAYANNISIVKAFSDFKKEQESNAELYAKKISSELDYK
NKRKFPEPFGSRQKVLKIDEIFVSKPERKKPSGALHEETFRKEEFYQ
SYGGKEGVLELKGKIRKVNKIVKNGDMFRVDIFKHKKTNKFYAVPIY
TMDFAKVLNPKAVARSKKGEIKDWILMDENYEFCSLYKDSLILIQTKD
MQEPEFVYNAFTSSTVSLIVSKHDNKFTLSKNQKILFKNANEKEVIAK
SIGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK.

[0228] The putative tracrRNA element for this CjCas9 is:

TATAATCTCATAAGAAATTTAAAAAGGGACTAAAAATAAGAGTTTGCG
GGACTCTGCGGGTTACAATCCCCATAAACCGCTTTTAAATTT

[0229] The Direct Repeat sequence is:

ATTTTACCATAAGAAATTTAAAAAGGGACTAAAAAC

[0230] The co-fold structure of the tracrRNA and direct repeat is provided in FIG. 6.

[0231] An example of a chimeric guide RNA for CjCas9 is:

NNNNNNNNNNNNNNNNNNNGUUUAGUCCCGAAAGGGACUAAAAUAA
AGAGUUUGCGGGACUCUGCGGGUUACAUAUCCCUAAAACCGCUUUU

[0232] Applicants have also optimized Cas9 guide RNA using in vitro methods. FIG. 18 shows data from the St1Cas9 chimeric guide RNA optimization in vitro.

[0233] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

Example 8

Sa sgRNA Optimization

[0234] Applicants designed five sgRNA variants for SaCas9 for an optimal truncated architecture with highest cleavage efficiency. In addition, the native direct repeat:tracr duplex system was tested alongside sgRNAs. Guides with indicated lengths were co-transfected with SaCas9 and tested in HEK 293FT cells for activity. A total of 100 ng sgRNA U6-PCR amplicon (or 50 ng of direct repeat and 50 ng of tracrRNA) and 400 ng of SaCas9 plasmid were co-transfected into 200,000 Hepal-6 mouse hepatocytes, and DNA was harvested 72-hours post-transfection for SURVEYOR analysis. The results are shown in FIG. 23.

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- [0281] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention.

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Lys

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 aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180
 atgcttaccg taacttgaaa gtatttcgat ttcttggett tatatatctt gtggaaagga 240
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gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag    60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga    120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat    180
atgcttaccg taacttgaaa gtatttcgat ttcttggett tatatatctt gtggaagga    240
cgaaacaccg ggttttagag ctatgctgtt ttgaatggtc caaaacggg tcttcgagaa    300
gacgttttag agctatgctg ttttgaatgg tcccaaac                                339

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<210> SEQ ID NO 42

<211> LENGTH: 309

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 42

```

gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag    60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga    120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat    180
atgcttaccg taacttgaaa gtatttcgat ttcttggett tatatatctt gtggaagga    240
cgaaacaccg ggtcttcgag aagacctgtt ttagagctag aaatagcaag taaaataag    300
gctagtccg                                309

```

<210> SEQ ID NO 43

<211> LENGTH: 1648

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 43

```

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
1           5           10          15
Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20          25          30
Gly Ile His Gly Val Pro Ala Ala Asp Lys Lys Tyr Ser Ile Gly Leu
35          40          45
Asp Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr
50          55          60
Lys Val Pro Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His
65          70          75          80
Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu
85          90          95
Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr
100         105         110
Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu
115        120        125

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Met	Ala	Lys	Val	Asp	Asp	Ser	Phe	Phe	His	Arg	Leu	Glu	Glu	Ser	Phe	130	135	140
Leu	Val	Glu	Glu	Asp	Lys	Lys	His	Glu	Arg	His	Pro	Ile	Phe	Gly	Asn	145	150	155
Ile	Val	Asp	Glu	Val	Ala	Tyr	His	Glu	Lys	Tyr	Pro	Thr	Ile	Tyr	His	165	170	175
Leu	Arg	Lys	Lys	Leu	Val	Asp	Ser	Thr	Asp	Lys	Ala	Asp	Leu	Arg	Leu	180	185	190
Ile	Tyr	Leu	Ala	Leu	Ala	His	Met	Ile	Lys	Phe	Arg	Gly	His	Phe	Leu	195	200	205
Ile	Glu	Gly	Asp	Leu	Asn	Pro	Asp	Asn	Ser	Asp	Val	Asp	Lys	Leu	Phe	210	215	220
Ile	Gln	Leu	Val	Gln	Thr	Tyr	Asn	Gln	Leu	Phe	Glu	Glu	Asn	Pro	Ile	225	230	235
Asn	Ala	Ser	Gly	Val	Asp	Ala	Lys	Ala	Ile	Leu	Ser	Ala	Arg	Leu	Ser	245	250	255
Lys	Ser	Arg	Arg	Leu	Glu	Asn	Leu	Ile	Ala	Gln	Leu	Pro	Gly	Glu	Lys	260	265	270
Lys	Asn	Gly	Leu	Phe	Gly	Asn	Leu	Ile	Ala	Leu	Ser	Leu	Gly	Leu	Thr	275	280	285
Pro	Asn	Phe	Lys	Ser	Asn	Phe	Asp	Leu	Ala	Glu	Asp	Ala	Lys	Leu	Gln	290	295	300
Leu	Ser	Lys	Asp	Thr	Tyr	Asp	Asp	Asp	Leu	Asp	Asn	Leu	Leu	Ala	Gln	305	310	315
Ile	Gly	Asp	Gln	Tyr	Ala	Asp	Leu	Phe	Leu	Ala	Ala	Lys	Asn	Leu	Ser	325	330	335
Asp	Ala	Ile	Leu	Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr	340	345	350
Lys	Ala	Pro	Leu	Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His	355	360	365
Gln	Asp	Leu	Thr	Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu	370	375	380
Lys	Tyr	Lys	Glu	Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly	385	390	395
Tyr	Ile	Asp	Gly	Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys	405	410	415
Pro	Ile	Leu	Glu	Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu	420	425	430
Asn	Arg	Glu	Asp	Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	435	440	445
Ile	Pro	His	Gln	Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	450	455	460
Gln	Glu	Asp	Phe	Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	465	470	475
Lys	Ile	Leu	Thr	Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	485	490	495
Gly	Asn	Ser	Arg	Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	500	505	510
Thr	Pro	Trp	Asn	Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	515	520	525
Ser	Phe	Ile	Glu	Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu			

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530					535					540						
Lys 545	Val	Leu	Pro	Lys 550	His	Ser	Leu	Leu	Tyr	Glu 555	Tyr	Phe	Thr	Val	Tyr 560	
Asn	Glu	Leu	Thr	Lys 565	Val	Lys	Tyr	Val	Thr 570	Glu	Gly	Met	Arg	Lys 575	Pro	
Ala	Phe	Leu	Ser	Gly 580	Glu	Gln	Lys	Lys 585	Ala	Ile	Val	Asp	Leu 590	Leu	Phe	
Lys	Thr	Asn	Arg	Lys 595	Val	Thr	Val 600	Lys	Gln	Leu	Lys	Glu 605	Asp	Tyr	Phe	
Lys	Lys 610	Ile	Glu	Cys	Phe 615	Asp	Ser	Val	Glu	Ile	Ser 620	Gly	Val	Glu	Asp	
Arg 625	Phe	Asn	Ala	Ser	Leu 630	Gly	Thr	Tyr	His	Asp 635	Leu	Leu	Lys	Ile	Ile 640	
Lys	Asp	Lys	Asp	Phe 645	Leu	Asp	Asn	Glu	Glu 650	Asn	Glu	Asp	Ile	Leu 655	Glu	
Asp	Ile	Val	Leu	Thr 660	Leu	Thr	Leu	Phe 665	Glu	Asp	Arg	Glu 670	Met	Ile	Glu	
Glu	Arg	Leu 675	Lys	Thr	Tyr	Ala	His 680	Leu	Phe	Asp	Asp	Lys 685	Val	Met	Lys	
Gln	Leu 690	Lys	Arg	Arg	Arg 695	Tyr	Thr	Gly	Trp	Gly	Arg 700	Leu	Ser	Arg	Lys	
Leu 705	Ile	Asn	Gly	Ile	Arg 710	Asp	Lys	Gln	Ser	Gly 715	Lys	Thr	Ile	Leu	Asp 720	
Phe	Leu	Lys	Ser	Asp 725	Gly	Phe	Ala	Asn	Arg 730	Asn	Phe	Met	Gln	Leu 735	Ile	
His	Asp	Asp	Ser	Leu 740	Thr	Phe	Lys	Glu 745	Asp	Ile	Gln	Lys 750	Ala	Gln	Val	
Ser	Gly	Gln 755	Gly	Asp	Ser	Leu	His 760	Glu	His	Ile	Ala	Asn 765	Leu	Ala	Gly	
Ser	Pro 770	Ala	Ile	Lys	Lys 775	Gly	Ile	Leu	Gln	Thr	Val 780	Lys	Val	Val	Asp	
Glu 785	Leu	Val	Lys	Val	Met 790	Gly	Arg	His	Lys	Pro 795	Glu	Asn	Ile	Val	Ile 800	
Glu	Met	Ala	Arg	Glu 805	Asn	Gln	Thr	Thr	Gln 810	Lys	Gly	Gln	Lys	Asn 815	Ser	
Arg	Glu	Arg	Met	Lys 820	Arg	Ile	Glu	Glu 825	Gly	Ile	Lys	Glu 830	Leu	Gly	Ser	
Gln	Ile	Leu 835	Lys	Glu	His	Pro	Val 840	Glu	Asn	Thr	Gln 845	Leu	Gln	Asn	Glu	
Lys 850	Leu	Tyr	Leu	Tyr	Tyr 855	Leu	Gln	Asn	Gly	Arg	Asp 860	Met	Tyr	Val	Asp	
Gln 865	Glu	Leu	Asp	Ile	Asn 870	Arg	Leu	Ser	Asp	Tyr 875	Asp	Val	Asp	His	Ile 880	
Val	Pro	Gln	Ser	Phe 885	Leu	Lys	Asp	Asp	Ser	Ile	Asp	Asn 890	Lys	Val	Leu 895	
Thr	Arg	Ser	Asp 900	Lys	Asn	Arg	Gly	Lys 905	Ser	Asp	Asn	Val 910	Pro	Ser	Glu	
Glu	Val	Val 915	Lys	Lys	Met	Lys	Asn 920	Tyr	Trp	Arg	Gln 925	Leu	Leu	Asn	Ala	
Lys 930	Leu	Ile	Thr	Gln	Arg 935	Lys	Phe	Asp	Asn	Leu	Thr 940	Lys	Ala	Glu	Arg	

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Gly	Gly	Leu	Ser	Glu	Leu	Asp	Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu	945	950	955	960
Val	Glu	Thr	Arg	Gln	Ile	Thr	Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser	965	970	975	
Arg	Met	Asn	Thr	Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	980	985	990	
Lys	Val	Ile	Thr	Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	995	1000	1005	
Phe	Gln	Phe	Tyr	Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala		1010	1015	1020	
His	Asp	Ala	Tyr	Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys		1025	1030	1035	
Lys	Tyr	Pro	Lys	Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys		1040	1045	1050	
Val	Tyr	Asp	Val	Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile		1055	1060	1065	
Gly	Lys	Ala	Thr	Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn		1070	1075	1080	
Phe	Phe	Lys	Thr	Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys		1085	1090	1095	
Arg	Pro	Leu	Ile	Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp		1100	1105	1110	
Asp	Lys	Gly	Arg	Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met		1115	1120	1125	
Pro	Gln	Val	Asn	Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly		1130	1135	1140	
Phe	Ser	Lys	Glu	Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu		1145	1150	1155	
Ile	Ala	Arg	Lys	Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe		1160	1165	1170	
Asp	Ser	Pro	Thr	Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val		1175	1180	1185	
Glu	Lys	Gly	Lys	Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu		1190	1195	1200	
Gly	Ile	Thr	Ile	Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile		1205	1210	1215	
Asp	Phe	Leu	Glu	Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu		1220	1225	1230	
Ile	Ile	Lys	Leu	Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly		1235	1240	1245	
Arg	Lys	Arg	Met	Leu	Ala	Ser	Ala	Gly	Glu	Leu	Gln	Lys	Gly	Asn		1250	1255	1260	
Glu	Leu	Ala	Leu	Pro	Ser	Lys	Tyr	Val	Asn	Phe	Leu	Tyr	Leu	Ala		1265	1270	1275	
Ser	His	Tyr	Glu	Lys	Leu	Lys	Gly	Ser	Pro	Glu	Asp	Asn	Glu	Gln		1280	1285	1290	
Lys	Gln	Leu	Phe	Val	Glu	Gln	His	Lys	His	Tyr	Leu	Asp	Glu	Ile		1295	1300	1305	
Ile	Glu	Gln	Ile	Ser	Glu	Phe	Ser	Lys	Arg	Val	Ile	Leu	Ala	Asp		1310	1315	1320	

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Ala	Asn	Leu	Asp	Lys	Val	Leu	Ser	Ala	Tyr	Asn	Lys	His	Arg	Asp
1325						1330					1335			
Lys	Pro	Ile	Arg	Glu	Gln	Ala	Glu	Asn	Ile	Ile	His	Leu	Phe	Thr
1340						1345					1350			
Leu	Thr	Asn	Leu	Gly	Ala	Pro	Ala	Ala	Phe	Lys	Tyr	Phe	Asp	Thr
1355						1360					1365			
Thr	Ile	Asp	Arg	Lys	Arg	Tyr	Thr	Ser	Thr	Lys	Glu	Val	Leu	Asp
1370						1375					1380			
Ala	Thr	Leu	Ile	His	Gln	Ser	Ile	Thr	Gly	Leu	Tyr	Glu	Thr	Arg
1385						1390					1395			
Ile	Asp	Leu	Ser	Gln	Leu	Gly	Gly	Asp	Ala	Ala	Ala	Val	Ser	Lys
1400						1405					1410			
Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu
1415						1420					1425			
Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly
1430						1435					1440			
Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys
1445						1450					1455			
Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr
1460						1465					1470			
Leu	Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met
1475						1480					1485			
Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val
1490						1495					1500			
Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr
1505						1510					1515			
Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile
1520						1525					1530			
Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly
1535						1540					1545			
His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met
1550						1555					1560			
Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg
1565						1570					1575			
His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln
1580						1585					1590			
Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn
1595						1600					1605			
His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu
1610						1615					1620			
Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly
1625						1630					1635			
Ile	Thr	Leu	Gly	Met	Asp	Glu	Leu	Tyr	Lys					
1640						1645								

<210> SEQ ID NO 44

<211> LENGTH: 1625

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

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<400> SEQUENCE: 44

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Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1      5      10      15
Gly Trp Ala Val Ile Thr Asp Glu Tyr Lys Val Pro Ser Lys Lys Phe
20      25      30
Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
35      40      45
Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50      55      60
Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65      70      75      80
Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
85      90      95
Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
100     105     110
His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
115     120     125
His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
130     135     140
Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145     150     155     160
Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
165     170     175
Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
180     185     190
Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
195     200     205
Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
210     215     220
Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Gly Leu Phe Gly Asn
225     230     235     240
Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
245     250     255
Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
260     265     270
Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
275     280     285
Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
290     295     300
Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305     310     315     320
Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
325     330     335
Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
340     345     350
Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
355     360     365
Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
370     375     380
Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385     390     395     400

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Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	Ile	Pro	His	Gln	Ile	His	Leu
				405					410					415	
Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	Gln	Glu	Asp	Phe	Tyr	Pro	Phe
			420					425					430		
Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	Lys	Ile	Leu	Thr	Phe	Arg	Ile
		435					440					445			
Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	Gly	Asn	Ser	Arg	Phe	Ala	Trp
	450					455					460				
Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	Thr	Pro	Trp	Asn	Phe	Glu	Glu
465					470					475				480	
Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	Ser	Phe	Ile	Glu	Arg	Met	Thr
				485					490					495	
Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu	Lys	Val	Leu	Pro	Lys	His	Ser
			500					505					510		
Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr	Asn	Glu	Leu	Thr	Lys	Val	Lys
		515					520					525			
Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro	Ala	Phe	Leu	Ser	Gly	Glu	Gln
	530					535					540				
Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe	Lys	Thr	Asn	Arg	Lys	Val	Thr
545					550					555				560	
Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe	Lys	Lys	Ile	Glu	Cys	Phe	Asp
				565					570					575	
Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp	Arg	Phe	Asn	Ala	Ser	Leu	Gly
		580						585					590		
Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile	Lys	Asp	Lys	Asp	Phe	Leu	Asp
		595					600					605			
Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu	Asp	Ile	Val	Leu	Thr	Leu	Thr
	610					615					620				
Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu	Glu	Arg	Leu	Lys	Thr	Tyr	Ala
625					630					635				640	
His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys	Gln	Leu	Lys	Arg	Arg	Arg	Tyr
				645					650					655	
Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys	Leu	Ile	Asn	Gly	Ile	Arg	Asp
		660						665					670		
Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp	Phe	Leu	Lys	Ser	Asp	Gly	Phe
		675					680					685			
Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile	His	Asp	Asp	Ser	Leu	Thr	Phe
	690					695					700				
Lys	Glu	Asp	Ile	Gln	Lys	Ala	Gln	Val	Ser	Gly	Gln	Gly	Asp	Ser	Leu
705					710					715				720	
His	Glu	His	Ile	Ala	Asn	Leu	Ala	Gly	Ser	Pro	Ala	Ile	Lys	Lys	Gly
				725					730					735	
Ile	Leu	Gln	Thr	Val	Lys	Val	Val	Asp	Glu	Leu	Val	Lys	Val	Met	Gly
			740					745					750		
Arg	His	Lys	Pro	Glu	Asn	Ile	Val	Ile	Glu	Met	Ala	Arg	Glu	Asn	Gln
		755					760					765			
Thr	Thr	Gln	Lys	Gly	Gln	Lys	Asn	Ser	Arg	Glu	Arg	Met	Lys	Arg	Ile
	770					775						780			
Glu	Glu	Gly	Ile	Lys	Glu	Leu	Gly	Ser	Gln	Ile	Leu	Lys	Glu	His	Pro
785					790					795				800	

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Val	Glu	Asn	Thr	Gln	Leu	Gln	Asn	Glu	Lys	Leu	Tyr	Leu	Tyr	Tyr	Leu	
				805					810					815		
Gln	Asn	Gly	Arg	Asp	Met	Tyr	Val	Asp	Gln	Glu	Leu	Asp	Ile	Asn	Arg	
			820					825					830			
Leu	Ser	Asp	Tyr	Asp	Val	Asp	His	Ile	Val	Pro	Gln	Ser	Phe	Leu	Lys	
		835					840					845				
Asp	Asp	Ser	Ile	Asp	Asn	Lys	Val	Leu	Thr	Arg	Ser	Asp	Lys	Asn	Arg	
	850					855					860					
Gly	Lys	Ser	Asp	Asn	Val	Pro	Ser	Glu	Glu	Val	Val	Lys	Lys	Met	Lys	
865					870					875					880	
Asn	Tyr	Trp	Arg	Gln	Leu	Leu	Asn	Ala	Lys	Leu	Ile	Thr	Gln	Arg	Lys	
				885					890					895		
Phe	Asp	Asn	Leu	Thr	Lys	Ala	Glu	Arg	Gly	Gly	Leu	Ser	Glu	Leu	Asp	
			900					905					910			
Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu	Val	Glu	Thr	Arg	Gln	Ile	Thr	
		915					920					925				
Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser	Arg	Met	Asn	Thr	Lys	Tyr	Asp	
	930					935					940					
Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val	Lys	Val	Ile	Thr	Leu	Lys	Ser	
945					950					955					960	
Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp	Phe	Gln	Phe	Tyr	Lys	Val	Arg	
				965					970					975		
Glu	Ile	Asn	Asn	Tyr	His	His	Ala	His	Asp	Ala	Tyr	Leu	Asn	Ala	Val	
			980					985					990			
Val	Gly	Thr	Ala	Leu	Ile	Lys	Lys	Tyr	Pro	Lys	Leu	Glu	Ser	Glu	Phe	
		995					1000					1005				
Val	Tyr	Gly	Asp	Tyr	Lys	Val	Tyr	Asp	Val	Arg	Lys	Met	Ile	Ala		
	1010					1015						1020				
Lys	Ser	Glu	Gln	Glu	Ile	Gly	Lys	Ala	Thr	Ala	Lys	Tyr	Phe	Phe		
	1025					1030						1035				
Tyr	Ser	Asn	Ile	Met	Asn	Phe	Phe	Lys	Thr	Glu	Ile	Thr	Leu	Ala		
	1040					1045						1050				
Asn	Gly	Glu	Ile	Arg	Lys	Arg	Pro	Leu	Ile	Glu	Thr	Asn	Gly	Glu		
	1055					1060						1065				
Thr	Gly	Glu	Ile	Val	Trp	Asp	Lys	Gly	Arg	Asp	Phe	Ala	Thr	Val		
	1070					1075						1080				
Arg	Lys	Val	Leu	Ser	Met	Pro	Gln	Val	Asn	Ile	Val	Lys	Lys	Thr		
	1085					1090						1095				
Glu	Val	Gln	Thr	Gly	Gly	Phe	Ser	Lys	Glu	Ser	Ile	Leu	Pro	Lys		
	1100					1105						1110				
Arg	Asn	Ser	Asp	Lys	Leu	Ile	Ala	Arg	Lys	Lys	Asp	Trp	Asp	Pro		
	1115					1120						1125				
Lys	Lys	Tyr	Gly	Gly	Phe	Asp	Ser	Pro	Thr	Val	Ala	Tyr	Ser	Val		
	1130					1135						1140				
Leu	Val	Val	Ala	Lys	Val	Glu	Lys	Gly	Lys	Ser	Lys	Lys	Leu	Lys		
	1145					1150						1155				
Ser	Val	Lys	Glu	Leu	Leu	Gly	Ile	Thr	Ile	Met	Glu	Arg	Ser	Ser		
	1160					1165						1170				
Phe	Glu	Lys	Asn	Pro	Ile	Asp	Phe	Leu	Glu	Ala	Lys	Gly	Tyr	Lys		
	1175					1180						1185				
Glu	Val	Lys	Lys	Asp	Leu	Ile	Ile	Lys	Leu	Pro	Lys	Tyr	Ser	Leu		

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1190	1195	1200
Phe Glu Leu Glu Asn Gly Arg Lys Arg Met Leu Ala Ser Ala Gly		
1205	1210	1215
Glu Leu Gln Lys Gly Asn Glu Leu Ala Leu Pro Ser Lys Tyr Val		
1220	1225	1230
Asn Phe Leu Tyr Leu Ala Ser His Tyr Glu Lys Leu Lys Gly Ser		
1235	1240	1245
Pro Glu Asp Asn Glu Gln Lys Gln Leu Phe Val Glu Gln His Lys		
1250	1255	1260
His Tyr Leu Asp Glu Ile Ile Glu Gln Ile Ser Glu Phe Ser Lys		
1265	1270	1275
Arg Val Ile Leu Ala Asp Ala Asn Leu Asp Lys Val Leu Ser Ala		
1280	1285	1290
Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn		
1295	1300	1305
Ile Ile His Leu Phe Thr Leu Thr Asn Leu Gly Ala Pro Ala Ala		
1310	1315	1320
Phe Lys Tyr Phe Asp Thr Thr Ile Asp Arg Lys Arg Tyr Thr Ser		
1325	1330	1335
Thr Lys Glu Val Leu Asp Ala Thr Leu Ile His Gln Ser Ile Thr		
1340	1345	1350
Gly Leu Tyr Glu Thr Arg Ile Asp Leu Ser Gln Leu Gly Gly Asp		
1355	1360	1365
Ala Ala Ala Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val		
1370	1375	1380
Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe		
1385	1390	1395
Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu		
1400	1405	1410
Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp		
1415	1420	1425
Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser		
1430	1435	1440
Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala		
1445	1450	1455
Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp		
1460	1465	1470
Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp		
1475	1480	1485
Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu		
1490	1495	1500
Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser		
1505	1510	1515
His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys		
1520	1525	1530
Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln		
1535	1540	1545
Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro		
1550	1555	1560
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu		
1565	1570	1575

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Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu
1580 1585 1590

Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr
1595 1600 1605

Lys Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys
1610 1615 1620

Lys Lys
1625

<210> SEQ ID NO 45

<211> LENGTH: 1664

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 45

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
1 5 10 15

Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20 25 30

Gly Ile His Gly Val Pro Ala Ala Asp Lys Lys Tyr Ser Ile Gly Leu
35 40 45

Asp Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr
50 55 60

Lys Val Pro Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His
65 70 75 80

Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu
85 90 95

Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr
100 105 110

Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu
115 120 125

Met Ala Lys Val Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser Phe
130 135 140

Leu Val Glu Glu Asp Lys Lys His Glu Arg His Pro Ile Phe Gly Asn
145 150 155 160

Ile Val Asp Glu Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr His
165 170 175

Leu Arg Lys Lys Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu
180 185 190

Ile Tyr Leu Ala Leu Ala His Met Ile Lys Phe Arg Gly His Phe Leu
195 200 205

Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu Phe
210 215 220

Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile
225 230 235 240

Asn Ala Ser Gly Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser
245 250 255

Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu Lys
260 265 270

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Lys	Asn	Gly	Leu	Phe	Gly	Asn	Leu	Ile	Ala	Leu	Ser	Leu	Gly	Leu	Thr	275	280	285
Pro	Asn	Phe	Lys	Ser	Asn	Phe	Asp	Leu	Ala	Glu	Asp	Ala	Lys	Leu	Gln	290	295	300
Leu	Ser	Lys	Asp	Thr	Tyr	Asp	Asp	Asp	Leu	Asp	Asn	Leu	Leu	Ala	Gln	305	310	315
Ile	Gly	Asp	Gln	Tyr	Ala	Asp	Leu	Phe	Leu	Ala	Ala	Lys	Asn	Leu	Ser	325	330	335
Asp	Ala	Ile	Leu	Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr	340	345	350
Lys	Ala	Pro	Leu	Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His	355	360	365
Gln	Asp	Leu	Thr	Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu	370	375	380
Lys	Tyr	Lys	Glu	Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly	385	390	395
Tyr	Ile	Asp	Gly	Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys	405	410	415
Pro	Ile	Leu	Glu	Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu	420	425	430
Asn	Arg	Glu	Asp	Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser	435	440	445
Ile	Pro	His	Gln	Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg	450	455	460
Gln	Glu	Asp	Phe	Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu	465	470	475
Lys	Ile	Leu	Thr	Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg	485	490	495
Gly	Asn	Ser	Arg	Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile	500	505	510
Thr	Pro	Trp	Asn	Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln	515	520	525
Ser	Phe	Ile	Glu	Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu	530	535	540
Lys	Val	Leu	Pro	Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr	545	550	555
Asn	Glu	Leu	Thr	Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro	565	570	575
Ala	Phe	Leu	Ser	Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe	580	585	590
Lys	Thr	Asn	Arg	Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe	595	600	605
Lys	Lys	Ile	Glu	Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp	610	615	620
Arg	Phe	Asn	Ala	Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile	625	630	635
Lys	Asp	Lys	Asp	Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu	645	650	655
Asp	Ile	Val	Leu	Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu	660	665	670
Glu	Arg	Leu	Lys	Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys			

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675	680	685
Gln Leu Lys Arg Arg Arg Tyr Thr Gly Trp Gly Arg Leu Ser Arg Lys 690 695 700		
Leu Ile Asn Gly Ile Arg Asp Lys Gln Ser Gly Lys Thr Ile Leu Asp 705 710 715 720		
Phe Leu Lys Ser Asp Gly Phe Ala Asn Arg Asn Phe Met Gln Leu Ile 725 730 735		
His Asp Asp Ser Leu Thr Phe Lys Glu Asp Ile Gln Lys Ala Gln Val 740 745 750		
Ser Gly Gln Gly Asp Ser Leu His Glu His Ile Ala Asn Leu Ala Gly 755 760 765		
Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val Asp 770 775 780		
Glu Leu Val Lys Val Met Gly Arg His Lys Pro Glu Asn Ile Val Ile 785 790 795 800		
Glu Met Ala Arg Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn Ser 805 810 815		
Arg Glu Arg Met Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly Ser 820 825 830		
Gln Ile Leu Lys Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn Glu 835 840 845		
Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly Arg Asp Met Tyr Val Asp 850 855 860		
Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp Tyr Asp Val Asp His Ile 865 870 875 880		
Val Pro Gln Ser Phe Leu Lys Asp Asp Ser Ile Asp Asn Lys Val Leu 885 890 895		
Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser Asp Asn Val Pro Ser Glu 900 905 910		
Glu Val Val Lys Lys Met Lys Asn Tyr Trp Arg Gln Leu Leu Asn Ala 915 920 925		
Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn Leu Thr Lys Ala Glu Arg 930 935 940		
Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly Phe Ile Lys Arg Gln Leu 945 950 955 960		
Val Glu Thr Arg Gln Ile Thr Lys His Val Ala Gln Ile Leu Asp Ser 965 970 975		
Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp Lys Leu Ile Arg Glu Val 980 985 990		
Lys Val Ile Thr Leu Lys Ser Lys Leu Val Ser Asp Phe Arg Lys Asp 995 1000 1005		
Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn Asn Tyr His His Ala 1010 1015 1020		
His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr Ala Leu Ile Lys 1025 1030 1035		
Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly Asp Tyr Lys 1040 1045 1050		
Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln Glu Ile 1055 1060 1065		
Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met Asn 1070 1075 1080		

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Phe	Phe	Lys	Thr	Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys
1085						1090					1095			
Arg	Pro	Leu	Ile	Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp
1100						1105					1110			
Asp	Lys	Gly	Arg	Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met
1115						1120					1125			
Pro	Gln	Val	Asn	Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly
1130						1135					1140			
Phe	Ser	Lys	Glu	Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu
1145						1150					1155			
Ile	Ala	Arg	Lys	Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe
1160						1165					1170			
Asp	Ser	Pro	Thr	Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val
1175						1180					1185			
Glu	Lys	Gly	Lys	Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu
1190						1195					1200			
Gly	Ile	Thr	Ile	Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile
1205						1210					1215			
Asp	Phe	Leu	Glu	Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu
1220						1225					1230			
Ile	Ile	Lys	Leu	Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly
1235						1240					1245			
Arg	Lys	Arg	Met	Leu	Ala	Ser	Ala	Gly	Glu	Leu	Gln	Lys	Gly	Asn
1250						1255					1260			
Glu	Leu	Ala	Leu	Pro	Ser	Lys	Tyr	Val	Asn	Phe	Leu	Tyr	Leu	Ala
1265						1270					1275			
Ser	His	Tyr	Glu	Lys	Leu	Lys	Gly	Ser	Pro	Glu	Asp	Asn	Glu	Gln
1280						1285					1290			
Lys	Gln	Leu	Phe	Val	Glu	Gln	His	Lys	His	Tyr	Leu	Asp	Glu	Ile
1295						1300					1305			
Ile	Glu	Gln	Ile	Ser	Glu	Phe	Ser	Lys	Arg	Val	Ile	Leu	Ala	Asp
1310						1315					1320			
Ala	Asn	Leu	Asp	Lys	Val	Leu	Ser	Ala	Tyr	Asn	Lys	His	Arg	Asp
1325						1330					1335			
Lys	Pro	Ile	Arg	Glu	Gln	Ala	Glu	Asn	Ile	Ile	His	Leu	Phe	Thr
1340						1345					1350			
Leu	Thr	Asn	Leu	Gly	Ala	Pro	Ala	Ala	Phe	Lys	Tyr	Phe	Asp	Thr
1355						1360					1365			
Thr	Ile	Asp	Arg	Lys	Arg	Tyr	Thr	Ser	Thr	Lys	Glu	Val	Leu	Asp
1370						1375					1380			
Ala	Thr	Leu	Ile	His	Gln	Ser	Ile	Thr	Gly	Leu	Tyr	Glu	Thr	Arg
1385						1390					1395			
Ile	Asp	Leu	Ser	Gln	Leu	Gly	Gly	Asp	Ala	Ala	Ala	Val	Ser	Lys
1400						1405					1410			
Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu
1415						1420					1425			
Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly
1430						1435					1440			
Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys
1445						1450					1455			

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Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr
1460						1465					1470			
Leu	Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met
1475						1480					1485			
Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val
1490						1495					1500			
Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr
1505						1510					1515			
Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile
1520						1525					1530			
Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly
1535						1540					1545			
His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met
1550						1555					1560			
Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg
1565						1570					1575			
His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln
1580						1585					1590			
Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn
1595						1600					1605			
His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu
1610						1615					1620			
Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly
1625						1630					1635			
Ile	Thr	Leu	Gly	Met	Asp	Glu	Leu	Tyr	Lys	Lys	Arg	Pro	Ala	Ala
1640						1645					1650			
Thr	Lys	Lys	Ala	Gly	Gln	Ala	Lys	Lys	Lys	Lys				
1655						1660								

<210> SEQ ID NO 46
 <211> LENGTH: 1423
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: source
 <223> OTHER INFORMATION: /note="Description of Artificial Sequence:
 Synthetic polypeptide"

<400> SEQUENCE: 46

Met	Asp	Tyr	Lys	Asp	His	Asp	Gly	Asp	Tyr	Lys	Asp	His	Asp	Ile	Asp
1				5					10					15	
Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Met	Ala	Pro	Lys	Lys	Lys	Arg	Lys	Val
		20						25					30		
Gly	Ile	His	Gly	Val	Pro	Ala	Ala	Asp	Lys	Lys	Tyr	Ser	Ile	Gly	Leu
		35					40					45			
Asp	Ile	Gly	Thr	Asn	Ser	Val	Gly	Trp	Ala	Val	Ile	Thr	Asp	Glu	Tyr
		50				55					60				
Lys	Val	Pro	Ser	Lys	Lys	Phe	Lys	Val	Leu	Gly	Asn	Thr	Asp	Arg	His
		65			70					75				80	
Ser	Ile	Lys	Lys	Asn	Leu	Ile	Gly	Ala	Leu	Leu	Phe	Asp	Ser	Gly	Glu
			85					90					95		
Thr	Ala	Glu	Ala	Thr	Arg	Leu	Lys	Arg	Thr	Ala	Arg	Arg	Arg	Tyr	Thr
		100					105						110		
Arg	Arg	Lys	Asn	Arg	Ile	Cys	Tyr	Leu	Gln	Glu	Ile	Phe	Ser	Asn	Glu

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115	120	125
Met Ala Lys Val Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser Phe 130 135 140		
Leu Val Glu Glu Asp Lys Lys His Glu Arg His Pro Ile Phe Gly Asn 145 150 155 160		
Ile Val Asp Glu Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr His 165 170 175		
Leu Arg Lys Lys Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu 180 185 190		
Ile Tyr Leu Ala Leu Ala His Met Ile Lys Phe Arg Gly His Phe Leu 195 200 205		
Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu Phe 210 215 220		
Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile 225 230 235 240		
Asn Ala Ser Gly Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser 245 250 255		
Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu Lys 260 265 270		
Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala Leu Ser Leu Gly Leu Thr 275 280 285		
Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala Glu Asp Ala Lys Leu Gln 290 295 300		
Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu Asp Asn Leu Leu Ala Gln 305 310 315 320		
Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu Ala Ala Lys Asn Leu Ser 325 330 335		
Asp Ala Ile Leu Leu Ser Asp Ile Leu Arg Val Asn Thr Glu Ile Thr 340 345 350		
Lys Ala Pro Leu Ser Ala Ser Met Ile Lys Arg Tyr Asp Glu His His 355 360 365		
Gln Asp Leu Thr Leu Leu Lys Ala Leu Val Arg Gln Gln Leu Pro Glu 370 375 380		
Lys Tyr Lys Glu Ile Phe Phe Asp Gln Ser Lys Asn Gly Tyr Ala Gly 385 390 395 400		
Tyr Ile Asp Gly Gly Ala Ser Gln Glu Glu Phe Tyr Lys Phe Ile Lys 405 410 415		
Pro Ile Leu Glu Lys Met Asp Gly Thr Glu Glu Leu Leu Val Lys Leu 420 425 430		
Asn Arg Glu Asp Leu Leu Arg Lys Gln Arg Thr Phe Asp Asn Gly Ser 435 440 445		
Ile Pro His Gln Ile His Leu Gly Glu Leu His Ala Ile Leu Arg Arg 450 455 460		
Gln Glu Asp Phe Tyr Pro Phe Leu Lys Asp Asn Arg Glu Lys Ile Glu 465 470 475 480		
Lys Ile Leu Thr Phe Arg Ile Pro Tyr Tyr Val Gly Pro Leu Ala Arg 485 490 495		
Gly Asn Ser Arg Phe Ala Trp Met Thr Arg Lys Ser Glu Glu Thr Ile 500 505 510		
Thr Pro Trp Asn Phe Glu Glu Val Val Asp Lys Gly Ala Ser Ala Gln 515 520 525		

Ser	Phe	Ile	Glu	Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu
530					535					540					
Lys	Val	Leu	Pro	Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr
545					550					555					
Asn	Glu	Leu	Thr	Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro
565					570					575					
Ala	Phe	Leu	Ser	Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe
580					585					590					
Lys	Thr	Asn	Arg	Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe
595					600					605					
Lys	Lys	Ile	Glu	Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp
610					615					620					
Arg	Phe	Asn	Ala	Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile
625					630					635					
Lys	Asp	Lys	Asp	Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu
645					650					655					
Asp	Ile	Val	Leu	Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu
660					665					670					
Glu	Arg	Leu	Lys	Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys
675					680					685					
Gln	Leu	Lys	Arg	Arg	Arg	Tyr	Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys
690					695					700					
Leu	Ile	Asn	Gly	Ile	Arg	Asp	Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp
705					710					715					
Phe	Leu	Lys	Ser	Asp	Gly	Phe	Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile
725					730					735					
His	Asp	Asp	Ser	Leu	Thr	Phe	Lys	Glu	Asp	Ile	Gln	Lys	Ala	Gln	Val
740					745					750					
Ser	Gly	Gln	Gly	Asp	Ser	Leu	His	Glu	His	Ile	Ala	Asn	Leu	Ala	Gly
755					760					765					
Ser	Pro	Ala	Ile	Lys	Lys	Gly	Ile	Leu	Gln	Thr	Val	Lys	Val	Val	Asp
770					775					780					
Glu	Leu	Val	Lys	Val	Met	Gly	Arg	His	Lys	Pro	Glu	Asn	Ile	Val	Ile
785					790					795					
Glu	Met	Ala	Arg	Glu	Asn	Gln	Thr	Thr	Gln	Lys	Gly	Gln	Lys	Asn	Ser
805					810					815					
Arg	Glu	Arg	Met	Lys	Arg	Ile	Glu	Glu	Gly	Ile	Lys	Glu	Leu	Gly	Ser
820					825					830					
Gln	Ile	Leu	Lys	Glu	His	Pro	Val	Glu	Asn	Thr	Gln	Leu	Gln	Asn	Glu
835					840					845					
Lys	Leu	Tyr	Leu	Tyr	Tyr	Leu	Gln	Asn	Gly	Arg	Asp	Met	Tyr	Val	Asp
850					855					860					
Gln	Glu	Leu	Asp	Ile	Asn	Arg	Leu	Ser	Asp	Tyr	Asp	Val	Asp	His	Ile
865					870					875					
Val	Pro	Gln	Ser	Phe	Leu	Lys	Asp	Asp	Ser	Ile	Asp	Asn	Lys	Val	Leu
885					890					895					
Thr	Arg	Ser	Asp	Lys	Asn	Arg	Gly	Lys	Ser	Asp	Asn	Val	Pro	Ser	Glu
900					905					910					
Glu	Val	Val	Lys	Lys	Met	Lys	Asn	Tyr	Trp	Arg	Gln	Leu	Leu	Asn	Ala
915					920					925					

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Lys	Leu	Ile	Thr	Gln	Arg	Lys	Phe	Asp	Asn	Leu	Thr	Lys	Ala	Glu	Arg
930						935						940			
Gly	Gly	Leu	Ser	Glu	Leu	Asp	Lys	Ala	Gly	Phe	Ile	Lys	Arg	Gln	Leu
945					950					955					960
Val	Glu	Thr	Arg	Gln	Ile	Thr	Lys	His	Val	Ala	Gln	Ile	Leu	Asp	Ser
				965					970					975	
Arg	Met	Asn	Thr	Lys	Tyr	Asp	Glu	Asn	Asp	Lys	Leu	Ile	Arg	Glu	Val
			980					985					990		
Lys	Val	Ile	Thr	Leu	Lys	Ser	Lys	Leu	Val	Ser	Asp	Phe	Arg	Lys	Asp
	995						1000					1005			
Phe	Gln	Phe	Tyr	Lys	Val	Arg	Glu	Ile	Asn	Asn	Tyr	His	His	Ala	
1010						1015					1020				
His	Asp	Ala	Tyr	Leu	Asn	Ala	Val	Val	Gly	Thr	Ala	Leu	Ile	Lys	
1025						1030					1035				
Lys	Tyr	Pro	Lys	Leu	Glu	Ser	Glu	Phe	Val	Tyr	Gly	Asp	Tyr	Lys	
1040						1045					1050				
Val	Tyr	Asp	Val	Arg	Lys	Met	Ile	Ala	Lys	Ser	Glu	Gln	Glu	Ile	
1055						1060					1065				
Gly	Lys	Ala	Thr	Ala	Lys	Tyr	Phe	Phe	Tyr	Ser	Asn	Ile	Met	Asn	
1070						1075					1080				
Phe	Phe	Lys	Thr	Glu	Ile	Thr	Leu	Ala	Asn	Gly	Glu	Ile	Arg	Lys	
1085						1090					1095				
Arg	Pro	Leu	Ile	Glu	Thr	Asn	Gly	Glu	Thr	Gly	Glu	Ile	Val	Trp	
1100						1105					1110				
Asp	Lys	Gly	Arg	Asp	Phe	Ala	Thr	Val	Arg	Lys	Val	Leu	Ser	Met	
1115						1120					1125				
Pro	Gln	Val	Asn	Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly	
1130						1135					1140				
Phe	Ser	Lys	Glu	Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu	
1145						1150					1155				
Ile	Ala	Arg	Lys	Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe	
1160						1165					1170				
Asp	Ser	Pro	Thr	Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val	
1175						1180					1185				
Glu	Lys	Gly	Lys	Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu	
1190						1195					1200				
Gly	Ile	Thr	Ile	Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile	
1205						1210					1215				
Asp	Phe	Leu	Glu	Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu	
1220						1225					1230				
Ile	Ile	Lys	Leu	Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly	
1235						1240					1245				
Arg	Lys	Arg	Met	Leu	Ala	Ser	Ala	Gly	Glu	Leu	Gln	Lys	Gly	Asn	
1250						1255					1260				
Glu	Leu	Ala	Leu	Pro	Ser	Lys	Tyr	Val	Asn	Phe	Leu	Tyr	Leu	Ala	
1265						1270					1275				
Ser	His	Tyr	Glu	Lys	Leu	Lys	Gly	Ser	Pro	Glu	Asp	Asn	Glu	Gln	
1280						1285					1290				
Lys	Gln	Leu	Phe	Val	Glu	Gln	His	Lys	His	Tyr	Leu	Asp	Glu	Ile	
1295						1300					1305				
Ile	Glu	Gln	Ile	Ser	Glu	Phe	Ser	Lys	Arg	Val	Ile	Leu	Ala	Asp	

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1310	1315	1320
Ala Asn Leu Asp Lys Val Leu Ser Ala Tyr Asn Lys His Arg Asp		
1325	1330	1335
Lys Pro Ile Arg Glu Gln Ala Glu Asn Ile Ile His Leu Phe Thr		
1340	1345	1350
Leu Thr Asn Leu Gly Ala Pro Ala Ala Phe Lys Tyr Phe Asp Thr		
1355	1360	1365
Thr Ile Asp Arg Lys Arg Tyr Thr Ser Thr Lys Glu Val Leu Asp		
1370	1375	1380
Ala Thr Leu Ile His Gln Ser Ile Thr Gly Leu Tyr Glu Thr Arg		
1385	1390	1395
Ile Asp Leu Ser Gln Leu Gly Gly Asp Lys Arg Pro Ala Ala Thr		
1400	1405	1410
Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys		
1415	1420	

<210> SEQ ID NO 47
 <211> LENGTH: 483
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: source
 <223> OTHER INFORMATION: /note="Description of Artificial Sequence:
 Synthetic polypeptide"

<400> SEQUENCE: 47

Met Phe Leu Phe Leu Ser Leu Thr Ser Phe Leu Ser Ser Ser Arg Thr		
1	5	10 15
Leu Val Ser Lys Gly Glu Glu Asp Asn Met Ala Ile Ile Lys Glu Phe		
20	25	30
Met Arg Phe Lys Val His Met Glu Gly Ser Val Asn Gly His Glu Phe		
35	40	45
Glu Ile Glu Gly Glu Gly Glu Gly Arg Pro Tyr Glu Gly Thr Gln Thr		
50	55	60
Ala Lys Leu Lys Val Thr Lys Gly Gly Pro Leu Pro Phe Ala Trp Asp		
65	70	75 80
Ile Leu Ser Pro Gln Phe Met Tyr Gly Ser Lys Ala Tyr Val Lys His		
85	90	95
Pro Ala Asp Ile Pro Asp Tyr Leu Lys Leu Ser Phe Pro Glu Gly Phe		
100	105	110
Lys Trp Glu Arg Val Met Asn Phe Glu Asp Gly Gly Val Val Thr Val		
115	120	125
Thr Gln Asp Ser Ser Leu Gln Asp Gly Glu Phe Ile Tyr Lys Val Lys		
130	135	140
Leu Arg Gly Thr Asn Phe Pro Ser Asp Gly Pro Val Met Gln Lys Lys		
145	150	155 160
Thr Met Gly Trp Glu Ala Ser Ser Glu Arg Met Tyr Pro Glu Asp Gly		
165	170	175
Ala Leu Lys Gly Glu Ile Lys Gln Arg Leu Lys Leu Lys Asp Gly Gly		
180	185	190
His Tyr Asp Ala Glu Val Lys Thr Thr Tyr Lys Ala Lys Lys Pro Val		
195	200	205
Gln Leu Pro Gly Ala Tyr Asn Val Asn Ile Lys Leu Asp Ile Thr Ser		
210	215	220

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His Asn Glu Asp Tyr Thr Ile Val Glu Gln Tyr Glu Arg Ala Glu Gly
 225 230 235 240
 Arg His Ser Thr Gly Gly Met Asp Glu Leu Tyr Lys Gly Ser Lys Gln
 245 250 255
 Leu Glu Glu Leu Leu Ser Thr Ser Phe Asp Ile Gln Phe Asn Asp Leu
 260 265 270
 Thr Leu Leu Glu Thr Ala Phe Thr His Thr Ser Tyr Ala Asn Glu His
 275 280 285
 Arg Leu Leu Asn Val Ser His Asn Glu Arg Leu Glu Phe Leu Gly Asp
 290 295 300
 Ala Val Leu Gln Leu Ile Ile Ser Glu Tyr Leu Phe Ala Lys Tyr Pro
 305 310 315 320
 Lys Lys Thr Glu Gly Asp Met Ser Lys Leu Arg Ser Met Ile Val Arg
 325 330 335
 Glu Glu Ser Leu Ala Gly Phe Ser Arg Phe Cys Ser Phe Asp Ala Tyr
 340 345 350
 Ile Lys Leu Gly Lys Gly Glu Glu Lys Ser Gly Gly Arg Arg Arg Asp
 355 360 365
 Thr Ile Leu Gly Asp Leu Phe Glu Ala Phe Leu Gly Ala Leu Leu Leu
 370 375 380
 Asp Lys Gly Ile Asp Ala Val Arg Arg Phe Leu Lys Gln Val Met Ile
 385 390 395 400
 Pro Gln Val Glu Lys Gly Asn Phe Glu Arg Val Lys Asp Tyr Lys Thr
 405 410 415
 Cys Leu Gln Glu Phe Leu Gln Thr Lys Gly Asp Val Ala Ile Asp Tyr
 420 425 430
 Gln Val Ile Ser Glu Lys Gly Pro Ala His Ala Lys Gln Phe Glu Val
 435 440 445
 Ser Ile Val Val Asn Gly Ala Val Leu Ser Lys Gly Leu Gly Lys Ser
 450 455 460
 Lys Lys Leu Ala Glu Gln Asp Ala Ala Lys Asn Ala Leu Ala Gln Leu
 465 470 475 480
 Ser Glu Val

<210> SEQ ID NO 48
 <211> LENGTH: 483
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <221> NAME/KEY: source
 <223> OTHER INFORMATION: /note="Description of Artificial Sequence:
 Synthetic polypeptide"

<400> SEQUENCE: 48

Met Lys Gln Leu Glu Glu Leu Leu Ser Thr Ser Phe Asp Ile Gln Phe
 1 5 10 15
 Asn Asp Leu Thr Leu Leu Glu Thr Ala Phe Thr His Thr Ser Tyr Ala
 20 25 30
 Asn Glu His Arg Leu Leu Asn Val Ser His Asn Glu Arg Leu Glu Phe
 35 40 45
 Leu Gly Asp Ala Val Leu Gln Leu Ile Ile Ser Glu Tyr Leu Phe Ala
 50 55 60
 Lys Tyr Pro Lys Lys Thr Glu Gly Asp Met Ser Lys Leu Arg Ser Met

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65	70				75				80			
Ile Val Arg Glu Glu Ser Leu Ala Gly Phe Ser Arg Phe Cys Ser Phe	85				90				95			
Asp Ala Tyr Ile Lys Leu Gly Lys Gly Glu Glu Lys Ser Gly Gly Arg	100				105				110			
Arg Arg Asp Thr Ile Leu Gly Asp Leu Phe Glu Ala Phe Leu Gly Ala	115				120				125			
Leu Leu Leu Asp Lys Gly Ile Asp Ala Val Arg Arg Phe Leu Lys Gln	130				135				140			
Val Met Ile Pro Gln Val Glu Lys Gly Asn Phe Glu Arg Val Lys Asp	145				150				155			
Tyr Lys Thr Cys Leu Gln Glu Phe Leu Gln Thr Lys Gly Asp Val Ala	165				170				175			
Ile Asp Tyr Gln Val Ile Ser Glu Lys Gly Pro Ala His Ala Lys Gln	180				185				190			
Phe Glu Val Ser Ile Val Val Asn Gly Ala Val Leu Ser Lys Gly Leu	195				200				205			
Gly Lys Ser Lys Lys Leu Ala Glu Gln Asp Ala Ala Lys Asn Ala Leu	210				215				220			
Ala Gln Leu Ser Glu Val Gly Ser Val Ser Lys Gly Glu Glu Asp Asn	225				230				235			
Met Ala Ile Ile Lys Glu Phe Met Arg Phe Lys Val His Met Glu Gly	245				250				255			
Ser Val Asn Gly His Glu Phe Glu Ile Glu Gly Glu Gly Glu Gly Arg	260				265				270			
Pro Tyr Glu Gly Thr Gln Thr Ala Lys Leu Lys Val Thr Lys Gly Gly	275				280				285			
Pro Leu Pro Phe Ala Trp Asp Ile Leu Ser Pro Gln Phe Met Tyr Gly	290				295				300			
Ser Lys Ala Tyr Val Lys His Pro Ala Asp Ile Pro Asp Tyr Leu Lys	305				310				315			
Leu Ser Phe Pro Glu Gly Phe Lys Trp Glu Arg Val Met Asn Phe Glu	325				330				335			
Asp Gly Gly Val Val Thr Val Thr Gln Asp Ser Ser Leu Gln Asp Gly	340				345				350			
Glu Phe Ile Tyr Lys Val Lys Leu Arg Gly Thr Asn Phe Pro Ser Asp	355				360				365			
Gly Pro Val Met Gln Lys Lys Thr Met Gly Trp Glu Ala Ser Ser Glu	370				375				380			
Arg Met Tyr Pro Glu Asp Gly Ala Leu Lys Gly Glu Ile Lys Gln Arg	385				390				395			
Leu Lys Leu Lys Asp Gly Gly His Tyr Asp Ala Glu Val Lys Thr Thr	405				410				415			
Tyr Lys Ala Lys Lys Pro Val Gln Leu Pro Gly Ala Tyr Asn Val Asn	420				425				430			
Ile Lys Leu Asp Ile Thr Ser His Asn Glu Asp Tyr Thr Ile Val Glu	435				440				445			
Gln Tyr Glu Arg Ala Glu Gly Arg His Ser Thr Gly Gly Met Asp Glu	450				455				460			
Leu Tyr Lys Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys	465				470				475			

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Lys Lys Lys

<210> SEQ ID NO 49
<211> LENGTH: 1423
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 49

Met Asp Tyr Lys Asp His Asp Gly Asp Tyr Lys Asp His Asp Ile Asp
1 5 10 15
Tyr Lys Asp Asp Asp Lys Met Ala Pro Lys Lys Lys Arg Lys Val
20 25 30
Gly Ile His Gly Val Pro Ala Ala Asp Lys Lys Tyr Ser Ile Gly Leu
35 40 45
Ala Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile Thr Asp Glu Tyr
50 55 60
Lys Val Pro Ser Lys Lys Phe Lys Val Leu Gly Asn Thr Asp Arg His
65 70 75 80
Ser Ile Lys Lys Asn Leu Ile Gly Ala Leu Leu Phe Asp Ser Gly Glu
85 90 95
Thr Ala Glu Ala Thr Arg Leu Lys Arg Thr Ala Arg Arg Arg Tyr Thr
100 105 110
Arg Arg Lys Asn Arg Ile Cys Tyr Leu Gln Glu Ile Phe Ser Asn Glu
115 120 125
Met Ala Lys Val Asp Asp Ser Phe Phe His Arg Leu Glu Glu Ser Phe
130 135 140
Leu Val Glu Glu Asp Lys Lys His Glu Arg His Pro Ile Phe Gly Asn
145 150 155 160
Ile Val Asp Glu Val Ala Tyr His Glu Lys Tyr Pro Thr Ile Tyr His
165 170 175
Leu Arg Lys Lys Leu Val Asp Ser Thr Asp Lys Ala Asp Leu Arg Leu
180 185 190
Ile Tyr Leu Ala Leu Ala His Met Ile Lys Phe Arg Gly His Phe Leu
195 200 205
Ile Glu Gly Asp Leu Asn Pro Asp Asn Ser Asp Val Asp Lys Leu Phe
210 215 220
Ile Gln Leu Val Gln Thr Tyr Asn Gln Leu Phe Glu Glu Asn Pro Ile
225 230 235 240
Asn Ala Ser Gly Val Asp Ala Lys Ala Ile Leu Ser Ala Arg Leu Ser
245 250 255
Lys Ser Arg Arg Leu Glu Asn Leu Ile Ala Gln Leu Pro Gly Glu Lys
260 265 270
Lys Asn Gly Leu Phe Gly Asn Leu Ile Ala Leu Ser Leu Gly Leu Thr
275 280 285
Pro Asn Phe Lys Ser Asn Phe Asp Leu Ala Glu Asp Ala Lys Leu Gln
290 295 300
Leu Ser Lys Asp Thr Tyr Asp Asp Asp Leu Asp Asn Leu Leu Ala Gln
305 310 315 320
Ile Gly Asp Gln Tyr Ala Asp Leu Phe Leu Ala Ala Lys Asn Leu Ser

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325								330					335				
Asp	Ala	Ile	Leu	Leu	Ser	Asp	Ile	Leu	Arg	Val	Asn	Thr	Glu	Ile	Thr		
340								345					350				
Lys	Ala	Pro	Leu	Ser	Ala	Ser	Met	Ile	Lys	Arg	Tyr	Asp	Glu	His	His		
355								360					365				
Gln	Asp	Leu	Thr	Leu	Leu	Lys	Ala	Leu	Val	Arg	Gln	Gln	Leu	Pro	Glu		
370								375					380				
Lys	Tyr	Lys	Glu	Ile	Phe	Phe	Asp	Gln	Ser	Lys	Asn	Gly	Tyr	Ala	Gly		
385								390					395				
Tyr	Ile	Asp	Gly	Gly	Ala	Ser	Gln	Glu	Glu	Phe	Tyr	Lys	Phe	Ile	Lys		
405								410					415				
Pro	Ile	Leu	Glu	Lys	Met	Asp	Gly	Thr	Glu	Glu	Leu	Leu	Val	Lys	Leu		
420								425					430				
Asn	Arg	Glu	Asp	Leu	Leu	Arg	Lys	Gln	Arg	Thr	Phe	Asp	Asn	Gly	Ser		
435								440					445				
Ile	Pro	His	Gln	Ile	His	Leu	Gly	Glu	Leu	His	Ala	Ile	Leu	Arg	Arg		
450								455					460				
Gln	Glu	Asp	Phe	Tyr	Pro	Phe	Leu	Lys	Asp	Asn	Arg	Glu	Lys	Ile	Glu		
465								470					475				
Lys	Ile	Leu	Thr	Phe	Arg	Ile	Pro	Tyr	Tyr	Val	Gly	Pro	Leu	Ala	Arg		
485								490					495				
Gly	Asn	Ser	Arg	Phe	Ala	Trp	Met	Thr	Arg	Lys	Ser	Glu	Glu	Thr	Ile		
500								505					510				
Thr	Pro	Trp	Asn	Phe	Glu	Glu	Val	Val	Asp	Lys	Gly	Ala	Ser	Ala	Gln		
515								520					525				
Ser	Phe	Ile	Glu	Arg	Met	Thr	Asn	Phe	Asp	Lys	Asn	Leu	Pro	Asn	Glu		
530								535					540				
Lys	Val	Leu	Pro	Lys	His	Ser	Leu	Leu	Tyr	Glu	Tyr	Phe	Thr	Val	Tyr		
545								550					555				
Asn	Glu	Leu	Thr	Lys	Val	Lys	Tyr	Val	Thr	Glu	Gly	Met	Arg	Lys	Pro		
565								570					575				
Ala	Phe	Leu	Ser	Gly	Glu	Gln	Lys	Lys	Ala	Ile	Val	Asp	Leu	Leu	Phe		
580								585					590				
Lys	Thr	Asn	Arg	Lys	Val	Thr	Val	Lys	Gln	Leu	Lys	Glu	Asp	Tyr	Phe		
595								600					605				
Lys	Lys	Ile	Glu	Cys	Phe	Asp	Ser	Val	Glu	Ile	Ser	Gly	Val	Glu	Asp		
610								615					620				
Arg	Phe	Asn	Ala	Ser	Leu	Gly	Thr	Tyr	His	Asp	Leu	Leu	Lys	Ile	Ile		
625								630					635				
Lys	Asp	Lys	Asp	Phe	Leu	Asp	Asn	Glu	Glu	Asn	Glu	Asp	Ile	Leu	Glu		
645								650					655				
Asp	Ile	Val	Leu	Thr	Leu	Thr	Leu	Phe	Glu	Asp	Arg	Glu	Met	Ile	Glu		
660								665					670				
Glu	Arg	Leu	Lys	Thr	Tyr	Ala	His	Leu	Phe	Asp	Asp	Lys	Val	Met	Lys		
675								680					685				
Gln	Leu	Lys	Arg	Arg	Arg	Tyr	Thr	Gly	Trp	Gly	Arg	Leu	Ser	Arg	Lys		
690								695					700				
Leu	Ile	Asn	Gly	Ile	Arg	Asp	Lys	Gln	Ser	Gly	Lys	Thr	Ile	Leu	Asp		
705								710					715				
Phe	Leu	Lys	Ser	Asp	Gly	Phe	Ala	Asn	Arg	Asn	Phe	Met	Gln	Leu	Ile		
725								730					735				

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His Asp Asp Ser Leu Thr Phe Lys Glu Asp Ile Gln Lys Ala Gln Val	740	745	750
Ser Gly Gln Gly Asp Ser Leu His Glu His Ile Ala Asn Leu Ala Gly	755	760	765
Ser Pro Ala Ile Lys Lys Gly Ile Leu Gln Thr Val Lys Val Val Asp	770	775	780
Glu Leu Val Lys Val Met Gly Arg His Lys Pro Glu Asn Ile Val Ile	785	790	795
Glu Met Ala Arg Glu Asn Gln Thr Thr Gln Lys Gly Gln Lys Asn Ser	805	810	815
Arg Glu Arg Met Lys Arg Ile Glu Glu Gly Ile Lys Glu Leu Gly Ser	820	825	830
Gln Ile Leu Lys Glu His Pro Val Glu Asn Thr Gln Leu Gln Asn Glu	835	840	845
Lys Leu Tyr Leu Tyr Tyr Leu Gln Asn Gly Arg Asp Met Tyr Val Asp	850	855	860
Gln Glu Leu Asp Ile Asn Arg Leu Ser Asp Tyr Asp Val Asp His Ile	865	870	875
Val Pro Gln Ser Phe Leu Lys Asp Asp Ser Ile Asp Asn Lys Val Leu	885	890	895
Thr Arg Ser Asp Lys Asn Arg Gly Lys Ser Asp Asn Val Pro Ser Glu	900	905	910
Glu Val Val Lys Lys Met Lys Asn Tyr Trp Arg Gln Leu Leu Asn Ala	915	920	925
Lys Leu Ile Thr Gln Arg Lys Phe Asp Asn Leu Thr Lys Ala Glu Arg	930	935	940
Gly Gly Leu Ser Glu Leu Asp Lys Ala Gly Phe Ile Lys Arg Gln Leu	945	950	955
Val Glu Thr Arg Gln Ile Thr Lys His Val Ala Gln Ile Leu Asp Ser	965	970	975
Arg Met Asn Thr Lys Tyr Asp Glu Asn Asp Lys Leu Ile Arg Glu Val	980	985	990
Lys Val Ile Thr Leu Lys Ser Lys Leu Val Ser Asp Phe Arg Lys Asp	995	1000	1005
Phe Gln Phe Tyr Lys Val Arg Glu Ile Asn Asn Tyr His His Ala	1010	1015	1020
His Asp Ala Tyr Leu Asn Ala Val Val Gly Thr Ala Leu Ile Lys	1025	1030	1035
Lys Tyr Pro Lys Leu Glu Ser Glu Phe Val Tyr Gly Asp Tyr Lys	1040	1045	1050
Val Tyr Asp Val Arg Lys Met Ile Ala Lys Ser Glu Gln Glu Ile	1055	1060	1065
Gly Lys Ala Thr Ala Lys Tyr Phe Phe Tyr Ser Asn Ile Met Asn	1070	1075	1080
Phe Phe Lys Thr Glu Ile Thr Leu Ala Asn Gly Glu Ile Arg Lys	1085	1090	1095
Arg Pro Leu Ile Glu Thr Asn Gly Glu Thr Gly Glu Ile Val Trp	1100	1105	1110
Asp Lys Gly Arg Asp Phe Ala Thr Val Arg Lys Val Leu Ser Met	1115	1120	1125

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Pro	Gln	Val	Asn	Ile	Val	Lys	Lys	Thr	Glu	Val	Gln	Thr	Gly	Gly
1130						1135					1140			
Phe	Ser	Lys	Glu	Ser	Ile	Leu	Pro	Lys	Arg	Asn	Ser	Asp	Lys	Leu
1145						1150					1155			
Ile	Ala	Arg	Lys	Lys	Asp	Trp	Asp	Pro	Lys	Lys	Tyr	Gly	Gly	Phe
1160						1165					1170			
Asp	Ser	Pro	Thr	Val	Ala	Tyr	Ser	Val	Leu	Val	Val	Ala	Lys	Val
1175						1180					1185			
Glu	Lys	Gly	Lys	Ser	Lys	Lys	Leu	Lys	Ser	Val	Lys	Glu	Leu	Leu
1190						1195					1200			
Gly	Ile	Thr	Ile	Met	Glu	Arg	Ser	Ser	Phe	Glu	Lys	Asn	Pro	Ile
1205						1210					1215			
Asp	Phe	Leu	Glu	Ala	Lys	Gly	Tyr	Lys	Glu	Val	Lys	Lys	Asp	Leu
1220						1225					1230			
Ile	Ile	Lys	Leu	Pro	Lys	Tyr	Ser	Leu	Phe	Glu	Leu	Glu	Asn	Gly
1235						1240					1245			
Arg	Lys	Arg	Met	Leu	Ala	Ser	Ala	Gly	Glu	Leu	Gln	Lys	Gly	Asn
1250						1255					1260			
Glu	Leu	Ala	Leu	Pro	Ser	Lys	Tyr	Val	Asn	Phe	Leu	Tyr	Leu	Ala
1265						1270					1275			
Ser	His	Tyr	Glu	Lys	Leu	Lys	Gly	Ser	Pro	Glu	Asp	Asn	Glu	Gln
1280						1285					1290			
Lys	Gln	Leu	Phe	Val	Glu	Gln	His	Lys	His	Tyr	Leu	Asp	Glu	Ile
1295						1300					1305			
Ile	Glu	Gln	Ile	Ser	Glu	Phe	Ser	Lys	Arg	Val	Ile	Leu	Ala	Asp
1310						1315					1320			
Ala	Asn	Leu	Asp	Lys	Val	Leu	Ser	Ala	Tyr	Asn	Lys	His	Arg	Asp
1325						1330					1335			
Lys	Pro	Ile	Arg	Glu	Gln	Ala	Glu	Asn	Ile	Ile	His	Leu	Phe	Thr
1340						1345					1350			
Leu	Thr	Asn	Leu	Gly	Ala	Pro	Ala	Ala	Phe	Lys	Tyr	Phe	Asp	Thr
1355						1360					1365			
Thr	Ile	Asp	Arg	Lys	Arg	Tyr	Thr	Ser	Thr	Lys	Glu	Val	Leu	Asp
1370						1375					1380			
Ala	Thr	Leu	Ile	His	Gln	Ser	Ile	Thr	Gly	Leu	Tyr	Glu	Thr	Arg
1385						1390					1395			
Ile	Asp	Leu	Ser	Gln	Leu	Gly	Gly	Asp	Lys	Arg	Pro	Ala	Ala	Thr
1400						1405					1410			
Lys	Lys	Ala	Gly	Gln	Ala	Lys	Lys	Lys	Lys					
1415						1420								

<210> SEQ ID NO 50

<211> LENGTH: 2012

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 50

gaatgctgcc ctcagacccg cttcctccct gtccttgtct gtccaaggag aatgaggtct 60

cactggtgga ttteggacta ccctgaggag ctggcacctg aggacaagg cccccacct 120

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gcccagctcc agcctctgat gaggggtggg agagagctac atgaggttgc taagaaagcc 180
tccccctgaag gagaccacac agtgtgtgag gttggagtct ctacgagcgg gttctgtgcc 240
cccagggata gtctggctgt ccaggcactg ctcttgatat aaacaccacc tctagttat 300
gaaaccatgc ccattctgcc tctctgtatg gaaaagagca tggggctggc cctgggggtg 360
gtgtccactt taggcctctgt gggagatcat gggaaccac gcagtgggtc ataggctctc 420
tcatttacta ctcacatcca ctctgtgaag aagcgattat gatctctcct ctagaaactc 480
gtagagtccc atgtctgccg gcttccagag cctgcactcc tccaccttg cttggctttg 540
ctggggctag aggagctagg atgcacagca gctctgtgac cctttgtttg agaggaacag 600
gaaaaccacc cttctctctg gccactgtg tcctcttctc gcctgccat cccctctctg 660
gaatgttaga cccatgggag cagctggta gaggggaccc cggcctggg cccctaacc 720
tatgtagcct cagtcttccc atcaggtctc cagctcagcc tgagtgttga ggccccagt 780
gctgctctgg gggcctcctg agtttctcat ctgtgcccct cctccttg cccagtgtaa 840
ggtgtggttc cagaaccgga ggacaaagta caaacggcag aagctggagg aggaagggcc 900
tgagtccgag cagaagaaga agggctccca tcacatcaac cggtgggcga ttgccacgaa 960
gcaggccaat ggggaggaca tcgatgtcac ctccaatgac aagcttgcta gcgggtggca 1020
accacaaacc cagcagggca gagtgtctct tgctgtctgg caggcccctg cgtgggcca 1080
agctggactc tggccactcc ctggccaggc tttggggagg cctggagtca tggccccaca 1140
gggcttgaag cccggggcgg ccattgacag agggacaagc aatgggctgg ctgaggcctg 1200
ggaccacttg gccttctcct cggagagcct gcctgcctgg gcggggccgc ccgccaccgc 1260
agcctcccag ctgctctcgg tgtctccaat ctcccttttg tttgatgca tttctgtttt 1320
aatttatttt ccaggcacca ctgtagttta gtgatccca gtgtcccct tccctatggg 1380
aataataaaa gtctctctct taatgacag ggcatccagc tccagccca gagcctggg 1440
tggttagattc cggtctctag gccagtggtg ggtggttaga gcaaacgct tcagggcctg 1500
ggagcctggg gtgggttact ggtggagggg gtcaagggtg attcattaac tcctctcttt 1560
tgttggggga ccctgtctc tacctcagc tccacagcag gaaacagg ctacacatag 1620
ggaagggcca tcctgtatct tgagggagga caggcccagg tctttcttaa cgtattgaga 1680
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ggagggaaca ccattcaca aggctgacgg ttccagtcg aagtcgtggg cccaccagga 1860
tgctcacctg tccttgagga accgtgggc aggttgagac tgcagagaca gggcttaagg 1920
ctgagcctgc aaccagtccc cagtgactca gggcctctc agccaagaa agagcaacgt 1980
gccaggggcc gctgagctct tgtgttcacc tg 2012

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<210> SEQ ID NO 51

<211> LENGTH: 1153

<212> TYPE: PRT

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polypeptide"

<400> SEQUENCE: 51

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Met	Lys	Arg	Pro	Ala	Ala	Thr	Lys	Lys	Ala	Gly	Gln	Ala	Lys	Lys	Lys	1	5	10	15
Lys	Ser	Asp	Leu	Val	Leu	Gly	Leu	Asp	Ile	Gly	Ile	Gly	Ser	Val	Gly	20	25	30	
Val	Gly	Ile	Leu	Asn	Lys	Val	Thr	Gly	Glu	Ile	Ile	His	Lys	Asn	Ser	35	40	45	
Arg	Ile	Phe	Pro	Ala	Ala	Gln	Ala	Glu	Asn	Asn	Leu	Val	Arg	Arg	Thr	50	55	60	
Asn	Arg	Gln	Gly	Arg	Arg	Leu	Ala	Arg	Arg	Lys	Lys	His	Arg	Arg	Val	65	70	75	80
Arg	Leu	Asn	Arg	Leu	Phe	Glu	Glu	Ser	Gly	Leu	Ile	Thr	Asp	Phe	Thr	85	90	95	
Lys	Ile	Ser	Ile	Asn	Leu	Asn	Pro	Tyr	Gln	Leu	Arg	Val	Lys	Gly	Leu	100	105	110	
Thr	Asp	Glu	Leu	Ser	Asn	Glu	Glu	Leu	Phe	Ile	Ala	Leu	Lys	Asn	Met	115	120	125	
Val	Lys	His	Arg	Gly	Ile	Ser	Tyr	Leu	Asp	Asp	Ala	Ser	Asp	Asp	Gly	130	135	140	
Asn	Ser	Ser	Val	Gly	Asp	Tyr	Ala	Gln	Ile	Val	Lys	Glu	Asn	Ser	Lys	145	150	155	160
Gln	Leu	Glu	Thr	Lys	Thr	Pro	Gly	Gln	Ile	Gln	Leu	Glu	Arg	Tyr	Gln	165	170	175	
Thr	Tyr	Gly	Gln	Leu	Arg	Gly	Asp	Phe	Thr	Val	Glu	Lys	Asp	Gly	Lys	180	185	190	
Lys	His	Arg	Leu	Ile	Asn	Val	Phe	Pro	Thr	Ser	Ala	Tyr	Arg	Ser	Glu	195	200	205	
Ala	Leu	Arg	Ile	Leu	Gln	Thr	Gln	Gln	Glu	Phe	Asn	Pro	Gln	Ile	Thr	210	215	220	
Asp	Glu	Phe	Ile	Asn	Arg	Tyr	Leu	Glu	Ile	Leu	Thr	Gly	Lys	Arg	Lys	225	230	235	240
Tyr	Tyr	His	Gly	Pro	Gly	Asn	Glu	Lys	Ser	Arg	Thr	Asp	Tyr	Gly	Arg	245	250	255	
Tyr	Arg	Thr	Ser	Gly	Glu	Thr	Leu	Asp	Asn	Ile	Phe	Gly	Ile	Leu	Ile	260	265	270	
Gly	Lys	Cys	Thr	Phe	Tyr	Pro	Asp	Glu	Phe	Arg	Ala	Ala	Lys	Ala	Ser	275	280	285	
Tyr	Thr	Ala	Gln	Glu	Phe	Asn	Leu	Leu	Asn	Asp	Leu	Asn	Asn	Leu	Thr	290	295	300	
Val	Pro	Thr	Glu	Thr	Lys	Lys	Leu	Ser	Lys	Glu	Gln	Lys	Asn	Gln	Ile	305	310	315	320
Ile	Asn	Tyr	Val	Lys	Asn	Glu	Lys	Ala	Met	Gly	Pro	Ala	Lys	Leu	Phe	325	330	335	
Lys	Tyr	Ile	Ala	Lys	Leu	Leu	Ser	Cys	Asp	Val	Ala	Asp	Ile	Lys	Gly	340	345	350	
Tyr	Arg	Ile	Asp	Lys	Ser	Gly	Lys	Ala	Glu	Ile	His	Thr	Phe	Glu	Ala	355	360	365	
Tyr	Arg	Lys	Met	Lys	Thr	Leu	Glu	Thr	Leu	Asp	Ile	Glu	Gln	Met	Asp	370	375	380	
Arg	Glu	Thr	Leu	Asp	Lys	Leu	Ala	Tyr	Val	Leu	Thr	Leu	Asn	Thr	Glu	385	390	395	400
Arg	Glu	Gly	Ile	Gln	Glu	Ala	Leu	Glu	His	Glu	Phe	Ala	Asp	Gly	Ser				

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405								410					415					
Phe	Ser	Gln	Lys	Gln	Val	Asp	Glu	Leu	Val	Gln	Phe	Arg	Lys	Ala	Asn			
420								425					430					
Ser	Ser	Ile	Phe	Gly	Lys	Gly	Trp	His	Asn	Phe	Ser	Val	Lys	Leu	Met			
435								440					445					
Met	Glu	Leu	Ile	Pro	Glu	Leu	Tyr	Glu	Thr	Ser	Glu	Glu	Gln	Met	Thr			
450								455					460					
Ile	Leu	Thr	Arg	Leu	Gly	Lys	Gln	Lys	Thr	Thr	Ser	Ser	Ser	Asn	Lys			
465	470								475					480				
Thr	Lys	Tyr	Ile	Asp	Glu	Lys	Leu	Leu	Thr	Glu	Glu	Ile	Tyr	Asn	Pro			
485								490					495					
Val	Val	Ala	Lys	Ser	Val	Arg	Gln	Ala	Ile	Lys	Ile	Val	Asn	Ala	Ala			
500								505					510					
Ile	Lys	Glu	Tyr	Gly	Asp	Phe	Asp	Asn	Ile	Val	Ile	Glu	Met	Ala	Arg			
515								520					525					
Glu	Thr	Asn	Glu	Asp	Asp	Glu	Lys	Lys	Ala	Ile	Gln	Lys	Ile	Gln	Lys			
530								535					540					
Ala	Asn	Lys	Asp	Glu	Lys	Asp	Ala	Ala	Met	Leu	Lys	Ala	Ala	Asn	Gln			
545	550								555					560				
Tyr	Asn	Gly	Lys	Ala	Glu	Leu	Pro	His	Ser	Val	Phe	His	Gly	His	Lys			
565								570					575					
Gln	Leu	Ala	Thr	Lys	Ile	Arg	Leu	Trp	His	Gln	Gln	Gly	Glu	Arg	Cys			
580								585					590					
Leu	Tyr	Thr	Gly	Lys	Thr	Ile	Ser	Ile	His	Asp	Leu	Ile	Asn	Asn	Ser			
595								600					605					
Asn	Gln	Phe	Glu	Val	Asp	His	Ile	Leu	Pro	Leu	Ser	Ile	Thr	Phe	Asp			
610								615					620					
Asp	Ser	Leu	Ala	Asn	Lys	Val	Leu	Val	Tyr	Ala	Thr	Ala	Asn	Gln	Glu			
625	630								635					640				
Lys	Gly	Gln	Arg	Thr	Pro	Tyr	Gln	Ala	Leu	Asp	Ser	Met	Asp	Asp	Ala			
645								650					655					
Trp	Ser	Phe	Arg	Glu	Leu	Lys	Ala	Phe	Val	Arg	Glu	Ser	Lys	Thr	Leu			
660								665					670					
Ser	Asn	Lys	Lys	Lys	Glu	Tyr	Leu	Leu	Thr	Glu	Glu	Asp	Ile	Ser	Lys			
675								680					685					
Phe	Asp	Val	Arg	Lys	Lys	Phe	Ile	Glu	Arg	Asn	Leu	Val	Asp	Thr	Arg			
690								695					700					
Tyr	Ala	Ser	Arg	Val	Val	Leu	Asn	Ala	Leu	Gln	Glu	His	Phe	Arg	Ala			
705	710								715					720				
His	Lys	Ile	Asp	Thr	Lys	Val	Ser	Val	Val	Arg	Gly	Gln	Phe	Thr	Ser			
725								730					735					
Gln	Leu	Arg	Arg	His	Trp	Gly	Ile	Glu	Lys	Thr	Arg	Asp	Thr	Tyr	His			
740								745					750					
His	His	Ala	Val	Asp	Ala	Leu	Ile	Ile	Ala	Ala	Ser	Ser	Gln	Leu	Asn			
755								760					765					
Leu	Trp	Lys	Lys	Gln	Lys	Asn	Thr	Leu	Val	Ser	Tyr	Ser	Glu	Asp	Gln			
770								775					780					
Leu	Leu	Asp	Ile	Glu	Thr	Gly	Glu	Leu	Ile	Ser	Asp	Asp	Glu	Tyr	Lys			
785	790								795					800				
Glu	Ser	Val	Phe	Lys	Ala	Pro	Tyr	Gln	His	Phe	Val	Asp	Thr	Leu	Lys			
805								810					815					

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Ser Lys Glu Phe Glu Asp Ser Ile Leu Phe Ser Tyr Gln Val Asp Ser
      820                      825                      830

Lys Phe Asn Arg Lys Ile Ser Asp Ala Thr Ile Tyr Ala Thr Arg Gln
      835                      840                      845

Ala Lys Val Gly Lys Asp Lys Ala Asp Glu Thr Tyr Val Leu Gly Lys
      850                      855                      860

Ile Lys Asp Ile Tyr Thr Gln Asp Gly Tyr Asp Ala Phe Met Lys Ile
865                      870                      875                      880

Tyr Lys Lys Asp Lys Ser Lys Phe Leu Met Tyr Arg His Asp Pro Gln
      885                      890                      895

Thr Phe Glu Lys Val Ile Glu Pro Ile Leu Glu Asn Tyr Pro Asn Lys
      900                      905                      910

Gln Ile Asn Glu Lys Gly Lys Glu Val Pro Cys Asn Pro Phe Leu Lys
      915                      920                      925

Tyr Lys Glu Glu His Gly Tyr Ile Arg Lys Tyr Ser Lys Lys Gly Asn
      930                      935                      940

Gly Pro Glu Ile Lys Ser Leu Lys Tyr Tyr Asp Ser Lys Leu Gly Asn
945                      950                      955                      960

His Ile Asp Ile Thr Pro Lys Asp Ser Asn Asn Lys Val Val Leu Gln
      965                      970                      975

Ser Val Ser Pro Trp Arg Ala Asp Val Tyr Phe Asn Lys Thr Thr Gly
      980                      985                      990

Lys Tyr Glu Ile Leu Gly Leu Lys Tyr Ala Asp Leu Gln Phe Glu Lys
      995                      1000                      1005

Gly Thr Gly Thr Tyr Lys Ile Ser Gln Glu Lys Tyr Asn Asp Ile
      1010                      1015                      1020

Lys Lys Lys Glu Gly Val Asp Ser Asp Ser Glu Phe Lys Phe Thr
      1025                      1030                      1035

Leu Tyr Lys Asn Asp Leu Leu Leu Val Lys Asp Thr Glu Thr Lys
      1040                      1045                      1050

Glu Gln Gln Leu Phe Arg Phe Leu Ser Arg Thr Met Pro Lys Gln
      1055                      1060                      1065

Lys His Tyr Val Glu Leu Lys Pro Tyr Asp Lys Gln Lys Phe Glu
      1070                      1075                      1080

Gly Gly Glu Ala Leu Ile Lys Val Leu Gly Asn Val Ala Asn Ser
      1085                      1090                      1095

Gly Gln Cys Lys Lys Gly Leu Gly Lys Ser Asn Ile Ser Ile Tyr
      1100                      1105                      1110

Lys Val Arg Thr Asp Val Leu Gly Asn Gln His Ile Ile Lys Asn
      1115                      1120                      1125

Glu Gly Asp Lys Pro Lys Leu Asp Phe Lys Arg Pro Ala Ala Thr
      1130                      1135                      1140

Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
      1145                      1150

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<210> SEQ ID NO 52
<211> LENGTH: 340
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic polynucleotide"

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<400> SEQUENCE: 52

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gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccg ttactttaat cttgcagaag ctacaagat aaggcttcat gccgaaatca     300
acaccctgtc attttatggc aggggtgttt cgttatttaa                          340
```

<210> SEQ ID NO 53

<211> LENGTH: 360

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (288)..(317)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 53

```
gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccg ggttttagag ctatgctgtt ttgaatggc ccaaaacnnn nnnnnnnnnn     300
nnnnnnnnnn nnnnnnngtt ttagagctat gctgttttga atggtcccaa aacttttttt     360
```

<210> SEQ ID NO 54

<211> LENGTH: 318

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (250)..(269)

<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 54

```
gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccn nnnnnnnnnn nnnnnnnnng ttttagagct agaaatagca agttaaata     300
aggctagtcc gttttttt                          318
```

<210> SEQ ID NO 55

<211> LENGTH: 325

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (250)..(269)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 55

gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccn nnnnnnnnnn nnnnnnnnng ttttagagct agaaatagca agttaaata      300
aggctagtcc gttatcattt ttttt                                     325

<210> SEQ ID NO 56
<211> LENGTH: 337
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (250)..(269)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 56

gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccn nnnnnnnnnn nnnnnnnnng ttttagagct agaaatagca agttaaata      300
aggctagtcc gttatcaact tgaaaaagtg tttttttt                        337

<210> SEQ ID NO 57
<211> LENGTH: 352
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (250)..(269)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 57

gagggcctat ttcccatgat tccttcatat ttgcatatac gatacaaggc tgtagagag      60
ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga     120
aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat     180
atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga     240
cgaaacaccn nnnnnnnnnn nnnnnnnnng ttttagagct agaaatagca agttaaata      300

```

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aggctagtagtcc gttatcaact tgaaaaagtg gcaccgagtc ggtgcttttt tt 352

```

<210> SEQ ID NO 58
<211> LENGTH: 5101
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic polynucleotide"

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<400> SEQUENCE: 58

```

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc ccgccccatt 60
gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 120
atgggtggag tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 180
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta 240
catgacctta tgggactttc ctacttgga gtacatctac gtattagtca tcgctattac 300
catggtcgag gtgagcccca cgttctgctt cactctcccc atctcccccc cctccccacc 360
cccaattttg tattttattha ttttttaatt attttgtgca gcgatggggg cggggggggg 420
ggggggggcg gcgccaggcg gggcgggcg gggcgagggg cggggcgggg cgaggcgagg 480
aggtgcggcg gcagccaatc agagcggcgc gctccgaaag tttcctttta tggcgaggcg 540
gcggcgggcg cgccctata aaaagcgaag cgcgcggcgg gcgggagtcg ctgcgacgct 600
gccttcgccc cgtgccccgc tcgcgcgcgc cctcgcgcgc cccgccccgg ccttgactga 660
cccggttact cccacagggt agcggggcgg acggcccttc tcctccgggc tgtaattagc 720
tgagcaagag gtaagggttt aagggatggt tgggtggtgg ggtattaatg tttaattacc 780
tggagcacct gcctgaaatc acttttttcc aggttggacc ggtgccacca tggactataa 840
ggaccacgac ggagactaca aggatcatga tattgattac aaagacgatg acgataagat 900
ggccccaaag aagaagcgga aggtcgggat ccacggagtc ccagcagccg acaagaagta 960
cagcatcggc ctggacatcg gcaccaactc tgtgggctgg gccgtgatca ccgacgagta 1020
caaggtgccc agcaagaat tcaagggtct gggcaacacc gaccggcaca gcatcaagaa 1080
gaacctgatc ggagccctgc tgttcgacag cggcgaaaaca gccgaggcca cccggctgaa 1140
gagaaccgcc agaagaagat acaccagacg gaagaaccgg atctgctatc tgcaagagat 1200
cttcagcaac gagatggcca aggtggacga cagcttcttc cacagactgg aagagtctt 1260
cctggtggaa gaggataaga agcacgagcg gcaccccatc ttcggcaaca tcgtggacga 1320
ggtggcctac cagcagaagt accccacat ctaccacctg agaagaaac tggtgacag 1380
caccgacaag gccgacctgc ggctgatcta tctggccttg gccacatga tcaagttccg 1440
gggcccactc ctgatcgagg gcgacctgaa ccccgacaac agcgacgtgg acaagctgtt 1500
catccagctg gtgcagacct acaaccagct gttcgaggaa aaccccatca acgccagcgg 1560
cgtggacgcc aaggccatcc tgtctgccag actgagcaag agcagacggc tggaaaatct 1620
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gctgagcaag gacacctacg acgacgacct ggacaacctg ctggcccaga tcggcgacca 1800
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gaagtacaaa gagattttct tcgaccagag caagaacggc tacgccggct acattgacgg	2040
cggagccagc caggaagagt tctacaagtt catcaagccc atcctggaaa agatggacgg	2100
caccgaggaa ctgctcgtga agctgaacag agaggacctg ctgcggaagc agcggacctt	2160
cgacaacggc agcatcccc accagatcca cctgggagag ctgcacgcca ttctgcggcg	2220
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cgacgcctac ctgaacgcgg tcgtgggaac cgccctgac aaaaagtacc ctaagctgga	3960
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cgagcaggaa atcggcaagg ctaccgcaa gtacttcttc tacagcaaca tcatgaactt	4080
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caccctgatc caccagagca tcaccggcct gtacgagaca cggatcgacc tgtctcagct 5040
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<210> SEQ ID NO 59
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

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<400> SEQUENCE: 59

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nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt tagaaataaa tcttcagaaa 60
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tcgttattta atttttt 137

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<210> SEQ ID NO 60
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
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<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

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<400> SEQUENCE: 60

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nnnnnnnnnn nnnnnnnnnn gttttgtac tctcagaaat gcagaagcta caaagataag 60
gcttcagcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttcgt tatttaattt 120

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ttt 123

<210> SEQ ID NO 61
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 61

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcagaaat gcagaagcta caaagataag 60

gcttcacgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttttt 110

<210> SEQ ID NO 62
<211> LENGTH: 137
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 62

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcaagatt tagaaataaa tcttcagaaa 60

gctacaaaaga taaggcttca tgccgaaatc aacaccctgt cattttatgg caggggtgtt 120

tcgttattta atttttt 137

<210> SEQ ID NO 63
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 63

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcagaaat gcagaagcta caaagataag 60

gcttcacgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttcgt tatttaattt 120

ttt 123

<210> SEQ ID NO 64
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

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<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 64

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcagaaat gcagaagcta caaagataag 60
gcttcacatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttttt 110

<210> SEQ ID NO 65
<211> LENGTH: 137
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 65

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcaagatt tagaaataaa tcttcagaaa 60
gctacaatga taaggcttca tgccgaaatc aacaccctgt cattttatgg caggggtgttt 120
tcgttatatta attttttt 137

<210> SEQ ID NO 66
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 66

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcagaaat gcagaagcta caatgataag 60
gcttcacatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttcgt tatttaattt 120
ttt 123

<210> SEQ ID NO 67
<211> LENGTH: 110
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 67

nnnnnnnnnn nnnnnnnnnn gttattgtac tctcagaaat gcagaagcta caatgataag 60
gcttcacatgcc gaaatcaaca ccctgtcatt ttatggcagg gtgttttttt 110

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<210> SEQ ID NO 68
<211> LENGTH: 107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 68

nnnnnnnnnn nnnnnnnnnn gttttagagc tgtggaaaca cagcgagtta aaataaggct 60
tagtccgtac tcaactgaa aaggtggcac cgattcgtg tttttt 107

<210> SEQ ID NO 69
<211> LENGTH: 4263
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 69

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tacagcatcg gcctggacat cggcaccaat agcgtgggct gggccgtgac caccgacaac 120
tacaagggtc ccagcaagaa aatgaagggtg ctgggcaaca cctccaagaa gtacatcaag 180
aaaaacctgc tggcggtgct gctgttcgac agcggcatta cagccgaggg cagacggctg 240
aagagaaccg ccagacggcg gtacaccccg cggagaaaca gaatcctgta tctgcaagag 300
atcttcagca ccgagatggc tacctcggac gacgccttct tccagcggct ggacgacagc 360
ttcttggtgc ccgacgacaa gcgggacagc aagtacccca tcttcggcaa cctggtggaa 420
gagaaggcct accacgacga gttccccacc atctaccacc tgagaaagta cctggccgac 480
agcaccaaga aggccgacct gagactggtg tatctggccc tggcccat gatcaagtac 540
cggggccact tcttgatcga gggcgagttc aacagcaaga acaacgacat ccagaagaac 600
ttccaggact tcttgacac ctacaacgcc atcttcgaga gcgacctgtc cctggaaaac 660
agcaagcagc tggaagagat cgtgaaggac aagatcagca agctggaaaa gaaggaccgc 720
atctgaagc tgttccccgg cgagaagaac agcggaatct tcagcgagtt tctgaagctg 780
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ggggccgact actttctgga aaaaatcgac cgcgaggatt tctcgcgaa gcagcggacc 1260
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aagcaggcca agttctaccc attctggcc aagaacaaag agcggatcga gaagatcctg 1380

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accttcgcga tcccttacta cgtgggcccc ctggccagag gcaacagcga ttttgctgg	1440
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gagtcacgcy ccgaggcctt catcaaccgg atgaccagct tcgacctgta cctgcccag	1560
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cagttcaact ccagcctgag cacataccac gacctgctga acattatcaa cgacaaagaa	1860
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cagagacagc tgggtgaaac ccggcagatc accaagcacg tggccagact gctggatgag	2880
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atcaatgact ttcaccacgc ccacgacgc tacctgaatg ccgtgggtggc ttccgcctg	3060
ctgaagaagt accctaagct ggaaccggag ttcgtgtacg gcgactacc caagtacaac	3120
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atcgagctgc ctaagtactc cctgttcgaa ctgagcgacg gctccagacg gatgctggcc 3720
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agccagaaat ttgtgaaact gctgtaccac gccaaagcga tctccaacac catcaatgag 3840
aaccaccgga aatacgtgga aaaccacaag aaagagtttg aggaactgtt ctactacatc 3900
ctggagttca acgagaacta tgtgggagcc aagaagaacg gcaaactgct gaactccgcc 3960
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gccaccctga tccaccagag cgtgaccggc ctgtacgaaa cccggatcga cctgggctaag 4200
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taa 4263

<210> SEQ ID NO 70
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 70

ggaaccattc ataacagcat agcaagttat aataaggcta gtccgttatc aacttgaaaa 60
agtggcaccg agtcggtgct tttt 84

<210> SEQ ID NO 71
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 71

gttatagagc tatgctgtta tgaatggctc caaaac 36

<210> SEQ ID NO 72
<211> LENGTH: 84
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 72

ggaaccattc aatacagcat agcaagttaa tataaggcta gtccgttatc aacttgaaaa 60
agtggcaccg agtcggtgct tttt 84

<210> SEQ ID NO 73
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 73

gtattagagc tatgctgtat tgaatgggcc caaaac 36

<210> SEQ ID NO 74
<211> LENGTH: 103
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 74

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cgttatcaac ttgaaaaagt ggcaccgagt cgggtgctttt ttt 103

<210> SEQ ID NO 75
<211> LENGTH: 103
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 75

nnnnnnnnnn nnnnnnnnnn gtattagagc tagaaatagc aagttaatat aaggctagtc 60

cgttatcaac ttgaaaaagt ggcaccgagt cgggtgctttt ttt 103

<210> SEQ ID NO 76
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 76

nnnnnnnnnn nnnnnnnnnn gttttagagc tatgctgttt tggaaacaaa acagcatagc 60

aagttaaaat aaggctagtc cgttatcaac ttgaaaaagt ggcaccgagt cgggtgctttt 120

ttt 123

<210> SEQ ID NO 77
<211> LENGTH: 123
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 77

nnnnnnnnnn nnnnnnnnnn gtattagagc tatgctgtat tggaacaac acagcatagc 60
aagttaatat aaggctagtc cggtatcaac ttgaaaaagt ggcaccgagt cgggtgctttt 120
ttt 123

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 78

gtcacctcca atgactaggg 20

<210> SEQ ID NO 79
<211> LENGTH: 984
<212> TYPE: PRT
<213> ORGANISM: Campylobacter jejuni

<400> SEQUENCE: 79

Met Ala Arg Ile Leu Ala Phe Asp Ile Gly Ile Ser Ser Ile Gly Trp
1 5 10 15
Ala Phe Ser Glu Asn Asp Glu Leu Lys Asp Cys Gly Val Arg Ile Phe
20 25 30
Thr Lys Val Glu Asn Pro Lys Thr Gly Glu Ser Leu Ala Leu Pro Arg
35 40 45
Arg Leu Ala Arg Ser Ala Arg Lys Arg Leu Ala Arg Arg Lys Ala Arg
50 55 60
Leu Asn His Leu Lys His Leu Ile Ala Asn Glu Phe Lys Leu Asn Tyr
65 70 75 80
Glu Asp Tyr Gln Ser Phe Asp Glu Ser Leu Ala Lys Ala Tyr Lys Gly
85 90 95
Ser Leu Ile Ser Pro Tyr Glu Leu Arg Phe Arg Ala Leu Asn Glu Leu
100 105 110
Leu Ser Lys Gln Asp Phe Ala Arg Val Ile Leu His Ile Ala Lys Arg
115 120 125
Arg Gly Tyr Asp Asp Ile Lys Asn Ser Asp Asp Lys Glu Lys Gly Ala
130 135 140
Ile Leu Lys Ala Ile Lys Gln Asn Glu Glu Lys Leu Ala Asn Tyr Gln
145 150 155 160
Ser Val Gly Glu Tyr Leu Tyr Lys Glu Tyr Phe Gln Lys Phe Lys Glu
165 170 175
Asn Ser Lys Glu Phe Thr Asn Val Arg Asn Lys Lys Glu Ser Tyr Glu
180 185 190
Arg Cys Ile Ala Gln Ser Phe Leu Lys Asp Glu Leu Lys Leu Ile Phe
195 200 205
Lys Lys Gln Arg Glu Phe Gly Phe Ser Phe Ser Lys Lys Phe Glu Glu
210 215 220
Glu Val Leu Ser Val Ala Phe Tyr Lys Arg Ala Leu Lys Asp Phe Ser

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225	230							235							240
His	Leu	Val	Gly	Asn	Cys	Ser	Phe	Phe	Thr	Asp	Glu	Lys	Arg	Ala	Pro
				245					250					255	
Lys	Asn	Ser	Pro	Leu	Ala	Phe	Met	Phe	Val	Ala	Leu	Thr	Arg	Ile	Ile
			260					265					270		
Asn	Leu	Leu	Asn	Asn	Leu	Lys	Asn	Thr	Glu	Gly	Ile	Leu	Tyr	Thr	Lys
	275						280					285			
Asp	Asp	Leu	Asn	Ala	Leu	Leu	Asn	Glu	Val	Leu	Lys	Asn	Gly	Thr	Leu
	290					295					300				
Thr	Tyr	Lys	Gln	Thr	Lys	Lys	Leu	Leu	Gly	Leu	Ser	Asp	Asp	Tyr	Glu
305					310					315					320
Phe	Lys	Gly	Glu	Lys	Gly	Thr	Tyr	Phe	Ile	Glu	Phe	Lys	Lys	Tyr	Lys
				325				330						335	
Glu	Phe	Ile	Lys	Ala	Leu	Gly	Glu	His	Asn	Leu	Ser	Gln	Asp	Asp	Leu
			340					345					350		
Asn	Glu	Ile	Ala	Lys	Asp	Ile	Thr	Leu	Ile	Lys	Asp	Glu	Ile	Lys	Leu
	355						360					365			
Lys	Lys	Ala	Leu	Ala	Lys	Tyr	Asp	Leu	Asn	Gln	Asn	Gln	Ile	Asp	Ser
	370					375					380				
Leu	Ser	Lys	Leu	Glu	Phe	Lys	Asp	His	Leu	Asn	Ile	Ser	Phe	Lys	Ala
385					390					395					400
Leu	Lys	Leu	Val	Thr	Pro	Leu	Met	Leu	Glu	Gly	Lys	Lys	Tyr	Asp	Glu
			405						410					415	
Ala	Cys	Asn	Glu	Leu	Asn	Leu	Lys	Val	Ala	Ile	Asn	Glu	Asp	Lys	Lys
			420					425					430		
Asp	Phe	Leu	Pro	Ala	Phe	Asn	Glu	Thr	Tyr	Tyr	Lys	Asp	Glu	Val	Thr
	435					440						445			
Asn	Pro	Val	Val	Leu	Arg	Ala	Ile	Lys	Glu	Tyr	Arg	Lys	Val	Leu	Asn
	450					455					460				
Ala	Leu	Leu	Lys	Lys	Tyr	Gly	Lys	Val	His	Lys	Ile	Asn	Ile	Glu	Leu
465					470					475					480
Ala	Arg	Glu	Val	Gly	Lys	Asn	His	Ser	Gln	Arg	Ala	Lys	Ile	Glu	Lys
				485					490					495	
Glu	Gln	Asn	Glu	Asn	Tyr	Lys	Ala	Lys	Lys	Asp	Ala	Glu	Leu	Glu	Cys
			500					505					510		
Glu	Lys	Leu	Gly	Leu	Lys	Ile	Asn	Ser	Lys	Asn	Ile	Leu	Lys	Leu	Arg
	515					520						525			
Leu	Phe	Lys	Glu	Gln	Lys	Glu	Phe	Cys	Ala	Tyr	Ser	Gly	Glu	Lys	Ile
	530					535					540				
Lys	Ile	Ser	Asp	Leu	Gln	Asp	Glu	Lys	Met	Leu	Glu	Ile	Asp	His	Ile
545					550					555					560
Tyr	Pro	Tyr	Ser	Arg	Ser	Phe	Asp	Asp	Ser	Tyr	Met	Asn	Lys	Val	Leu
			565						570					575	
Val	Phe	Thr	Lys	Gln	Asn	Gln	Glu	Lys	Leu	Asn	Gln	Thr	Pro	Phe	Glu
			580					585					590		
Ala	Phe	Gly	Asn	Asp	Ser	Ala	Lys	Trp	Gln	Lys	Ile	Glu	Val	Leu	Ala
		595					600					605			
Lys	Asn	Leu	Pro	Thr	Lys	Lys	Gln	Lys	Arg	Ile	Leu	Asp	Lys	Asn	Tyr
	610					615					620				
Lys	Asp	Lys	Glu	Gln	Lys	Asn	Phe	Lys	Asp	Arg	Asn	Leu	Asn	Asp	Thr
625					630					635					640

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Arg Tyr Ile Ala Arg Leu Val Leu Asn Tyr Thr Lys Asp Tyr Leu Asp
      645                      650                      655

Phe Leu Pro Leu Ser Asp Asp Glu Asn Thr Lys Leu Asn Asp Thr Gln
      660                      665                      670

Lys Gly Ser Lys Val His Val Glu Ala Lys Ser Gly Met Leu Thr Ser
      675                      680                      685

Ala Leu Arg His Thr Trp Gly Phe Ser Ala Lys Asp Arg Asn Asn His
      690                      695                      700

Leu His His Ala Ile Asp Ala Val Ile Ile Ala Tyr Ala Asn Asn Ser
      705                      710                      715                      720

Ile Val Lys Ala Phe Ser Asp Phe Lys Lys Glu Gln Glu Ser Asn Ser
      725                      730                      735

Ala Glu Leu Tyr Ala Lys Lys Ile Ser Glu Leu Asp Tyr Lys Asn Lys
      740                      745                      750

Arg Lys Phe Phe Glu Pro Phe Ser Gly Phe Arg Gln Lys Val Leu Asp
      755                      760                      765

Lys Ile Asp Glu Ile Phe Val Ser Lys Pro Glu Arg Lys Lys Pro Ser
      770                      775                      780

Gly Ala Leu His Glu Glu Thr Phe Arg Lys Glu Glu Glu Phe Tyr Gln
      785                      790                      795                      800

Ser Tyr Gly Gly Lys Glu Gly Val Leu Lys Ala Leu Glu Leu Gly Lys
      805                      810                      815

Ile Arg Lys Val Asn Gly Lys Ile Val Lys Asn Gly Asp Met Phe Arg
      820                      825                      830

Val Asp Ile Phe Lys His Lys Lys Thr Asn Lys Phe Tyr Ala Val Pro
      835                      840                      845

Ile Tyr Thr Met Asp Phe Ala Leu Lys Val Leu Pro Asn Lys Ala Val
      850                      855                      860

Ala Arg Ser Lys Lys Gly Glu Ile Lys Asp Trp Ile Leu Met Asp Glu
      865                      870                      875                      880

Asn Tyr Glu Phe Cys Phe Ser Leu Tyr Lys Asp Ser Leu Ile Leu Ile
      885                      890                      895

Gln Thr Lys Asp Met Gln Glu Pro Glu Phe Val Tyr Tyr Asn Ala Phe
      900                      905                      910

Thr Ser Ser Thr Val Ser Leu Ile Val Ser Lys His Asp Asn Lys Phe
      915                      920                      925

Glu Thr Leu Ser Lys Asn Gln Lys Ile Leu Phe Lys Asn Ala Asn Glu
      930                      935                      940

Lys Glu Val Ile Ala Lys Ser Ile Gly Ile Gln Asn Leu Lys Val Phe
      945                      950                      955                      960

Glu Lys Tyr Ile Val Ser Ala Leu Gly Glu Val Thr Lys Ala Glu Phe
      965                      970                      975

Arg Gln Arg Glu Asp Phe Lys Lys
      980

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<210> SEQ ID NO 80

<211> LENGTH: 91

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

 <223> OTHER INFORMATION: /note="Description of Artificial Sequence:
 Synthetic oligonucleotide"

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<400> SEQUENCE: 80

tataatctca taagaaattt aaaaaggagac taaaataaag agtttgcggg actctgcggg 60

gttacaatcc cctaaaaccg cttttaaaat t 91

<210> SEQ ID NO 81

<211> LENGTH: 36

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 81

atattaccat aaagaaattt aaaaaggagac taaaac 36

<210> SEQ ID NO 82

<211> LENGTH: 95

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<220> FEATURE:

<221> NAME/KEY: modified_base

<222> LOCATION: (1)..(20)

<223> OTHER INFORMATION: a, c, u, g, unknown or other

<400> SEQUENCE: 82

nnnnnnnnnn nnnnnnnnnn guuuuagucc cgaaaggagac uaaaaaaaag aguuugcggg 60

acucugcggg guuacaaucc ccuaaaaccg cuuuu 95

<210> SEQ ID NO 83

<211> LENGTH: 69

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 83

gucaccucca augacuaggg guuuuagagc uagaaaauagc aaguuaaaau aaggcuaguc 60

cguuuuuuuu 69

<210> SEQ ID NO 84

<211> LENGTH: 69

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 84

gacaucgaug uccuccccau guuuuagagc uagaaaauagc aaguuaaaau aaggcuaguc 60

cguuuuuuuu 69

<210> SEQ ID NO 85

<211> LENGTH: 69

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic oligonucleotide"

<400> SEQUENCE: 85

gaguccgagc agaagaagaa guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc      60
cguuuuuuu                                     69

<210> SEQ ID NO 86
<211> LENGTH: 69
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic oligonucleotide"

<400> SEQUENCE: 86

ggggccgaga uuggguguuc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc      60
cguuuuuuu                                     69

<210> SEQ ID NO 87
<211> LENGTH: 69
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic oligonucleotide"

<400> SEQUENCE: 87

guggcgagag gggccgagau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc      60
cguuuuuuu                                     69

<210> SEQ ID NO 88
<211> LENGTH: 76
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic oligonucleotide"

<400> SEQUENCE: 88

gucaccucca augacuaggg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc      60
cguaucuuuuuuuuuuu                                     76

<210> SEQ ID NO 89
<211> LENGTH: 76
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
        Synthetic oligonucleotide"

<400> SEQUENCE: 89

gacaucgaug uccuccccau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc      60
cguaucuuuuuuuuuuu                                     76

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<210> SEQ ID NO 90	
<211> LENGTH: 76	
<212> TYPE: RNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<221> NAME/KEY: source	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic oligonucleotide"	
<400> SEQUENCE: 90	
gaguccgagc agaagaagaa guuuuagagc uagaaaagc aaguuuuuuu aaggcuaguc	60
cguaaucauu uuuuuu	76
<210> SEQ ID NO 91	
<211> LENGTH: 76	
<212> TYPE: RNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<221> NAME/KEY: source	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic oligonucleotide"	
<400> SEQUENCE: 91	
ggggccgaga ugggguguuc guuuuagagc uagaaaagc aaguuuuuuu aaggcuaguc	60
cguaaucauu uuuuuu	76
<210> SEQ ID NO 92	
<211> LENGTH: 76	
<212> TYPE: RNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<221> NAME/KEY: source	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic oligonucleotide"	
<400> SEQUENCE: 92	
guggcgagag gggccgagau guuuuagagc uagaaaagc aaguuuuuuu aaggcuaguc	60
cguaaucauu uuuuuu	76
<210> SEQ ID NO 93	
<211> LENGTH: 88	
<212> TYPE: RNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<221> NAME/KEY: source	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic oligonucleotide"	
<400> SEQUENCE: 93	
gucaccucca augacuaggg guuuuagagc uagaaaagc aaguuuuuuu aaggcuaguc	60
cguaaucaac uagaaaaagu guuuuuuu	88
<210> SEQ ID NO 94	
<211> LENGTH: 88	
<212> TYPE: RNA	
<213> ORGANISM: Artificial Sequence	
<220> FEATURE:	
<221> NAME/KEY: source	
<223> OTHER INFORMATION: /note="Description of Artificial Sequence: Synthetic oligonucleotide"	
<400> SEQUENCE: 94	

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gacaucgaug uccuccccau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu guuuuuuu 88

<210> SEQ ID NO 95

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 95

gaguccgagc agaagaagaa guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu guuuuuuu 88

<210> SEQ ID NO 96

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 96

ggggcgaga uuggguguuc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu guuuuuuu 88

<210> SEQ ID NO 97

<211> LENGTH: 88

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 97

guggcgagag gggccgagau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu guuuuuuu 88

<210> SEQ ID NO 98

<211> LENGTH: 103

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 98

gucaccucca augacuaggg guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu ggcaccgagu cggugcuuuu uu 103

<210> SEQ ID NO 99

<211> LENGTH: 103

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 99

gacaucgaug uccuccccau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu uuu 103

<210> SEQ ID NO 100

<211> LENGTH: 103

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 100

gaguccgagc agaagaagaa guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu uuu 103

<210> SEQ ID NO 101

<211> LENGTH: 103

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 101

ggggccgaga uuggguguuc guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu uuu 103

<210> SEQ ID NO 102

<211> LENGTH: 103

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 102

guggcgagag gggccgagau guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguuaucaac uugaaaaagu ggcaccgagu cggugcuuuu uuu 103

<210> SEQ ID NO 103

<211> LENGTH: 102

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 103

gttttagagc tatgctgttt tgaatggtcc caaacggaa gggcctgagt ccgagcagaa 60

gaagaagttt tagagctatg ctgttttgaa tggteccaaa ac 102

<210> SEQ ID NO 104

<211> LENGTH: 100

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 104
cggaggacaa agtacaacg gcagaagctg gaggaggaag ggcttgagtc cgagcagaag 60
aagaagggt cccatcacat caaccggtgg cgcattgccca 100

<210> SEQ ID NO 105
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 105
agctggagga ggaagggcct ggtccgagc agaagaagaa gggtccccc 50

<210> SEQ ID NO 106
<211> LENGTH: 30
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 106
gaguccgagc agaagaagaa guuuuagagc 30

<210> SEQ ID NO 107
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 107
agctggagga ggaagggcct ggtccgagc agaagagaag gggtcccat 49

<210> SEQ ID NO 108
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 108
ctggaggagg aagggcctga gtccgagcag aagaagaagg gctcccatca cat 53

<210> SEQ ID NO 109
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 109
ctggaggagg aagggcctga gtccgagcag aagaagaagg gctcccatc at 52

<210> SEQ ID NO 110
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 110
ctggaggagg aagggcctga gtccgagcag aagaagaagg gggtcccatc acat 54

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<210> SEQ ID NO 111
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 111

ctggaggagg aagggcctga gtccgagcag aagaagggt cccatcacat 50

<210> SEQ ID NO 112
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 112

ctggaggagg aagggcctga gcccgagcag aagggtccc atcacat 47

<210> SEQ ID NO 113
<211> LENGTH: 66
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Combined DNA/RNA
Molecule: Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 113

nnnnnnnnnn nnnnnnnnnn guuuuagagc uagaaauagc aaguuaaaau aaggctagtc 60

cguuuu 66

<210> SEQ ID NO 114
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 114

gaguccgagc agaagaagaa 20

<210> SEQ ID NO 115
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 115

gacaucgaug uccucccau 20

<210> SEQ ID NO 116
<211> LENGTH: 20

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 116

gucaccucca augacuaggg 20

<210> SEQ ID NO 117
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 117

auugggguguu cagggcagag 20

<210> SEQ ID NO 118
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 118

gugggcgagag gggccgagau 20

<210> SEQ ID NO 119
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 119

ggggccgaga uuggguguuc 20

<210> SEQ ID NO 120
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 120

gugccauuag cuaaugcau 20

<210> SEQ ID NO 121
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

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<400> SEQUENCE: 121

guaccaccca caggugccag

20

<210> SEQ ID NO 122

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 122

gaaagccucu gggccaggaa

20

<210> SEQ ID NO 123

<211> LENGTH: 48

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 123

ctggaggagg aagggcctga gtccgagcag aagaagaagg gctcccat

48

<210> SEQ ID NO 124

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 124

gaguccgagc agaagaagau

20

<210> SEQ ID NO 125

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 125

gaguccgagc agaagaagua

20

<210> SEQ ID NO 126

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 126

gaguccgagc agaagaacaa

20

<210> SEQ ID NO 127

<211> LENGTH: 20

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 127

gaguccgagc agaagaugaa 20

<210> SEQ ID NO 128
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 128

gaguccgagc agaaguagaa 20

<210> SEQ ID NO 129
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 129

gaguccgagc agaugaagaa 20

<210> SEQ ID NO 130
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 130

gaguccgagc acaagaagaa 20

<210> SEQ ID NO 131
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 131

gaguccgagg agaagaagaa 20

<210> SEQ ID NO 132
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 132

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gaguccgugc agaagaagaa 20

<210> SEQ ID NO 133
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 133

gagucggagc agaagaagaa 20

<210> SEQ ID NO 134
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 134

gagaccgagc agaagaagaa 20

<210> SEQ ID NO 135
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 135

aatgacaagc ttgctagcgg tggg 24

<210> SEQ ID NO 136
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 136

aaaacggaag ggcctgagtc cgagcagaag aagaagttt 39

<210> SEQ ID NO 137
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 137

aaacaggggc cgagattggg tggtcagggc agaggtttt 39

<210> SEQ ID NO 138
<211> LENGTH: 38

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

<400> SEQUENCE: 138

aaaacggaag ggcttgagtc cgagcagaag aagaagtt          38

<210> SEQ ID NO 139
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

<400> SEQUENCE: 139

aacggagggga ggggcacaga tgagaaactc agggtttttag      40

<210> SEQ ID NO 140
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 140

agcccttctt cttctgctcg gactcaggcc cttcctcc          38

<210> SEQ ID NO 141
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 141

caggaggaggga ggggcacaga tgagaaactc aggaggcccc      40

<210> SEQ ID NO 142
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

<400> SEQUENCE: 142

ggcaatgcgc caccggttga tgtgatggga gcccttctag gagggcccca gacgagccac  60
tggggcctca acactcaggc          80

<210> SEQ ID NO 143
<211> LENGTH: 98
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

<400> SEQUENCE: 143

ggacgaaaca ccggaacat tcaaaacagc atagcaagtt aaaataaggc tagtccgtta  60
tcaacttgaa aaagtggcac cgagtcggtg cttttttt          98

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<210> SEQ ID NO 144
<211> LENGTH: 186
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 144

ggacgaaaca ccggtagtat taagtattgt tttatggctg ataaatttct ttgaatttct 60
ccttgattat ttgttataaa agttataaaa taatcttggt ggaaccattc aaaacagcat 120
agcaagttaa aataaggcta gtccgttata aactgaaaa agtggcaccg agtcggtgct 180
tttttt 186

<210> SEQ ID NO 145
<211> LENGTH: 46
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: a, c, u, g, unknown or other

<400> SEQUENCE: 145

nnnnnnnnnn nnnnnnnnng uuauuguacu cucaagauuu auuuuu 46

<210> SEQ ID NO 146
<211> LENGTH: 91
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 146

guuacuuaaa ucuugcagaa gcuacaaaga uaaggcuuca ugccgaaauc aacaccuguu 60
cauuuuuagg caggguuuuu ucguuuuuu a 91

<210> SEQ ID NO 147
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 147

ttttctagtg ctgagtttct gtgactctc tacattctac ttctctgtgt ttctgtatac 60
tacctcctcc 70

<210> SEQ ID NO 148
<211> LENGTH: 122
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 148

ggaggaaggg cctgagtccg agcagaagaa gaagggtccc catcacatca accggtggcg 60

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cattgccacg aagcaggcca atggggagga catcgatgtc acctccaatg actagggtgg 120

gc 122

<210> SEQ ID NO 149
<211> LENGTH: 48
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (3)..(32)
<223> OTHER INFORMATION: a, c, u, g, unknown or other

<400> SEQUENCE: 149

acnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnguuuuaga gcuaugcu 48

<210> SEQ ID NO 150
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Combined DNA/RNA
Molecule: Synthetic oligonucleotide"

<400> SEQUENCE: 150

agcauagcaa guaaaaaaua ggctaguccg uuaucaacuu gaaaaagugg caccgagucg 60

gugcuuu 67

<210> SEQ ID NO 151
<211> LENGTH: 62
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, u, g, unknown or other

<400> SEQUENCE: 151

nnnnnnnnnn nnnnnnnnnn guuuuagagc uagaaaauagc aaguuaaaau aaggcuaguc 60

cg 62

<210> SEQ ID NO 152
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 152

tgaatggtcc caaaacggaa gggcctgagt ccgagcagaa gaagaagttt tagagctatg 60

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ctgttttgaa tgg 73

<210> SEQ ID NO 153
<211> LENGTH: 99
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, u, g, unknown or other

<400> SEQUENCE: 153

nnnnnnnnnn nnnnnnnnnn guuuuagagc uagaaauagc aaguuaaaau aaggcuaguc 60

cguaaucaac uugaaaaagu ggcaccgagu cggugcuuu 99

<210> SEQ ID NO 154
<211> LENGTH: 127
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 154

guuuuuguc ucuaagauu uaaguaacug uacaacguua cuuaaaucuu gcagaagcua 60

caaagauaag gcuucaugcc gaaaucaaca ccugucauu uuauggcagg guguuuucgu 120

uauuuuaa 127

<210> SEQ ID NO 155
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 155

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt taagtaactg tacaac 56

<210> SEQ ID NO 156
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 156

gttacttaaa tcttgagaa gctacaaaga taaggcttca tgccgaaatc aacaccctgt 60

cattttatgg cagggtgttt tcgttattha a 91

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<210> SEQ ID NO 157
<211> LENGTH: 134
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 157

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt taaggaaact aaatcttgca 60
gaagctacaa agataaggct tcatgccgaa atcaacaccc tgtcatttta tggcagggtg 120
ttttcgttat ttaa 134

<210> SEQ ID NO 158
<211> LENGTH: 131
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 158

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt tagaaataaa tcttcagaaa 60
gctacaaaga taaggcttca tgccgaaatc aacaccctgt cattttatgg cagggtgttt 120
tcgttattta a 131

<210> SEQ ID NO 159
<211> LENGTH: 125
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 159

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatg aaaatcttgc agaagctaca 60
aagataaggc ttcattgccg aatcaacacc ctgtcatttt atggcagggt gttttcgtaa 120
tttaa 125

<210> SEQ ID NO 160
<211> LENGTH: 112
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base

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<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 160

nnnnnnnnnn nnnnnnnnnn gtttttgtac tctgaaaaga agctacaaag ataaggcttc 60
atgccgaaat caacaccctg tcattttatg gcagggtggt ttcgttat tt aa 112

<210> SEQ ID NO 161
<211> LENGTH: 107
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 161

nnnnnnnnnn nnnnnnnnnn gtttttgtac tgaaaagcta caaagataag gcttcatgcc 60
gaaatcaaca ccctgtcatt ttatggcagg gtgttttcgt tatttaa 107

<210> SEQ ID NO 162
<211> LENGTH: 108
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 162

nnnnnnnnnn nnnnnnnnnn gtttttgtac tctcaagatt tagaaataaa tcttgcagaa 60
gctacaaaga taaggcttca tgccgaaatc aacaccctgt cattttat 108

<210> SEQ ID NO 163
<211> LENGTH: 86
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 163

nnnnnnnnnn nnnnnnnnnn gtttttgtac tctcaagatt tagaaataaa tcttgcagaa 60
gctacaaaga taaggcttca tgccga 86

<210> SEQ ID NO 164
<211> LENGTH: 79
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 164

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt tagaaataaa tcttcagaa      60
gctacaaaga taaggcttc                                              79

<210> SEQ ID NO 165
<211> LENGTH: 73
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (1)..(20)
<223> OTHER INFORMATION: a, c, t, g, unknown or other

<400> SEQUENCE: 165

nnnnnnnnnn nnnnnnnnnn gttttgtac tctcaagatt tagaaataaa tcttcagaa      60
gctacaaaga taa                                              73

<210> SEQ ID NO 166
<211> LENGTH: 125
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic polynucleotide"

<400> SEQUENCE: 166

guuuuagucc cuuuuuuuuu uucuuuauug uaaaauuuaa aucucauaag aaauuuuuuu      60
agggacuuaa auaaaagauu ugcgggacuc ugcggggguu caaucccuua aaaccgcuuu    120
uaaaa                                              125

<210> SEQ ID NO 167
<211> LENGTH: 91
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

<400> SEQUENCE: 167

guuacuuuaa ucuugcagaa gcuacaaaga uaaggcuuca ugccgaauc aacaccugu      60
cauuuuauug caggguuuu ucguuuuuu a                                              91

<210> SEQ ID NO 168
<211> LENGTH: 56
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
    Synthetic oligonucleotide"

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<400> SEQUENCE: 168

gggacucaac caagucauuc guuuuuguac ucucaagauu uaaguaacug uacaac 56

<210> SEQ ID NO 169

<211> LENGTH: 147

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 169

gggacucaac caagucauuc guuuuuguac ucucaagauu uaaguaacug uacaacguua 60

cuuaaaucuu gcagaagcua caaagauaag gcuucaugcc gaaaaucaaca ccugucuuu 120

uuauggcagg guguuuucgu uauuuuaa 147

<210> SEQ ID NO 170

<211> LENGTH: 70

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 170

cuugcagaag cuacaaagau aaggcuucau gccgaaaauca acaccuguc auuuuauggc 60

aggguuuuuu 70

<210> SEQ ID NO 171

<211> LENGTH: 42

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 171

gggacucaac caagucauuc guuuuuguac ucucaagauu ua 42

<210> SEQ ID NO 172

<211> LENGTH: 112

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 172

gggacucaac caagucauuc guuuuuguac ucucaagauu uacuugcaga agcuacaaag 60

auaaggcuuc augccgaaaau caacaccug ucauuuuuug gcaggguuuu uu 112

<210> SEQ ID NO 173

<211> LENGTH: 116

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 173

gggacucaac caagucauuc guuuuuguac ucucaagauu uagaaacuug cagaagcuac 60

aaagauaagg cuucaugccg aaaucaacac ccugucauuu uauggcaggg uguuuu 116

<210> SEQ ID NO 174

<211> LENGTH: 116

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 174

gggacucaac caagucauuc guuuuuguac ucucaagauu uagaaacuug cagaagcuac 60

aaagauaagg cuucaugccg aaaucaacac ccugucauuu uauggcaggg uguuuu 116

<210> SEQ ID NO 175

<211> LENGTH: 102

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<221> NAME/KEY: source

<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 175

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caacacccug ucauuuuuug gcaggguuguu uucguuuuu aa 102

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ggct 64

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ucgucaacuu guuggcgaga uuuuuuuu                                         147

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What is claimed is:

1. An engineered, non-naturally occurring Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-CRISPR associated (Cas) (CRISPR-Cas) vector system comprising one or more vectors comprising:

a) a first regulatory element operably linked to one or more nucleotide sequences encoding one or more CRISPR-Cas system polynucleotide sequences comprising a guide sequence, a tracr RNA, and a tracr mate sequence, wherein the guide sequence hybridizes with one or more target sequences in polynucleotide loci in a eukaryotic cell,

b) a second regulatory element operably linked to a nucleotide sequence encoding a Type II Cas9 protein, wherein components (a) and (b) are located on same or different vectors of the system,

wherein the CRISPR-Cas system comprises two or more nuclear localization signals (NLSs) expressed with the nucleotide sequence encoding the Cas9 protein, whereby the one or more guide sequences target the one or more polynucleotide loci in a eukaryotic cell and the Cas9 protein cleaves the one or more polynucleotide loci, whereby the sequence of the one or more polynucleotide loci is modified.

2. An engineered, non-naturally occurring Type II CRISPR-Cas vector system according to claim 1,

wherein the Cas9 protein is mutated with respect to a corresponding wild type Cas9 protein such that the mutated protein is a nickase that lacks the ability to cleave one strand of a target polynucleotide,

whereby the one or more guide sequences target the one or more polynucleotide loci in a eukaryotic cell and the Cas9 protein cleaves only one strand of the polynucleotide loci, whereby the sequence of the one or more polynucleotide loci is modified.

3. The system of claim 1 or 2, wherein the vectors are viral vectors.

4. The system of claim 3, wherein the viral vectors are retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors.

5. The system of any of claims 2-4 wherein the Cas9 protein comprises one or more mutations in the RuvC I, RuvC II or RuvC III catalytic domains.

6. The system of any of claims 2-4 wherein the Cas9 protein comprises a mutation selected from the group consisting of D10A, H840A, N854A and N863A with reference to the position numbering of a *Streptococcus pyogenes* Cas9 (SpCas9) protein.

7. The system of any preceding claim, wherein at least one NLS is at or near amino-terminus of the Cas9 protein and/or at least one NLS is at or near carboxy terminus of the Cas9 protein.

8. The system of claim 7, wherein at least one NLS is at or near amino-terminus of the Cas9 protein and at least one NLS is at or near carboxy terminus of the Cas9 protein.

9. The system of any preceding claim, wherein the one or more CRISPR-Cas system polynucleotide sequences comprise a guide sequence fused to a trans-activating cr (tracr) sequence.

10. The system of any preceding claim, wherein the CRISPR-Cas system polynucleotide sequence is a chimeric RNA comprising the guide sequence, the tracr sequence, and a tracr mate sequence.

11. The system of any preceding claim, wherein the eukaryotic cell is a mammalian cell or a human cell.

12. The system of any preceding claim, wherein the Cas9 protein is codon optimized for expression in a eukaryotic cell.

13. Use of the system of any of claims 1 to 12 for genome engineering, provided the use does not comprise a process for modifying the germ line genetic identity of human beings, and provided that said use is not a method for treatment of the human or animal body by surgery or therapy.

14. The use of claim 13 wherein the genome engineering comprises modifying a target polynucleotide in a eukaryotic cell, modifying expression of a polynucleotide in a eukaryotic cell, generating a model eukaryotic cell comprising a mutated disease gene, or knocking out a gene.

15. The use of claim 13 wherein the use further comprises repairing said cleaved target polynucleotide by inserting an exogenous template polynucleotide, wherein said repair results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide.

16. The use of claim 13 wherein the use further comprises editing said cleaved target polynucleotide by inserting an exogenous template polynucleotide, wherein said edit results in a mutation comprising an insertion, deletion, or substitution of one or more nucleotides of said target polynucleotide.

17. The use of claim 15 or 16 wherein the inserting is by homologous recombination.

18. Use of the system of any of claims 1 to 12 in the production of a non-human transgenic animal or transgenic plant.

* * * * *