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DESCRIPTION

Description

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of United States Provisional Application No. 62,140,454, filed March 31, 2015, United States Provisional Application No. 62,210,451, filed August 27, 2015, and United States Provisional Application No. 62,240,359 filed October 12, 2015.

INTRODUCTION

[0002] The instant disclosure relates to the use of engineered proteins with DNA binding proteins exhibiting genome specificity and in particular a catalytically inactive Cas9 (CRISPR (clustered regularly interspaced short palindromic repeats) protein) attached by a linker with a viral integrase (e.g. HIV or MMTV integrases) in order to deliver a DNA sequence of interest (or gene of interest) to a targeted site in a genome of a cell or organism. The use of a Cas9 that is inactive for its function in cutting DNA will allow us to use the Cas9 proteins ability to target DNA by the use of RNA guides (gRNA) without causing DNA breaks as intended in other systems for homologous recombination. The use of zinc finger proteins or TALE (engineered proteins that bind specific sequences of DNA) attached to the viral integrase is also disclosed. The system may be used for laboratory and therapeutic purposes. For example, donor DNA containing the gene(s) of interested can be easily introduced into host genome without the potential of off target cuts through conventional methods. Donor DNA can be engineered to facilitate "knock out" strategies as well. A new strategy for improving the specificity of Cas9 targeting is also discussed. This strategy uses surface bound dCas9 (Cas9 that is inactive for its DNA cutting ability) along with guide RNAs and genomic DNA in an assay to find which guide RNAs provide specific targeting of the Cas9. This will be especially important in in vivo applications of CRISPR/Cas9 and overcome limitations of the current in silico prediction models, although it may also be used in conjunction with in silico prediction models to make an educated determination of which gRNAs will be used in the assay.

BACKGROUND

[0003] Current advances in genome sequencing techniques and analysis methods have significantly accelerated the ability to catalog and map genetic/genomic factors that are

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. Genome-editing techniques such as designer zinc fingers, transcription activator-like effectors (TALEs), CRISPR/Cas9 or meganucleases are available for producing targeted genome perturbations, there remains a need for new genome engineering technologies that will allow the incorporation of DNA sequences (including full gene sequences) into a specific location in a given genome. This will allow for the production of cell lines or transgenic organisms that express an engineered gene or for the replacement of dysfunctional genes in a subject in need thereof.

[0004] Integrases are viral proteins that allow for the insertion of viral nucleic acids into a host genome (mammalian, human, mouse, rat, monkey, frog, fish, plant (including crop plants and experimental plants like Arabidopsis), laboratory or biomedical cell lines or primary cell cultures, C. elegans, fly (Drosophila), etc.). Integrases use DNA binding proteins of the host to bring the integrase in association with the host genome in order to incorporate the viral nucleic acid sequence into the host genome. Integrases are found in a retrovirus such as HIV (human immunodeficiency virus). Integrases depend on sequences on viral genes to insert their genome into host DNA. Leavitt et al (Journal of Biological Chemistry, 1993, volume 268, pages 2113-2119) examined the function of HIV1 integrase by using site directed mutagenesis and in vitro studies. Leavitt also indicates sequence of U5 and U3 HIV1 att sites that are important for the integration of HIV1 DNA (created after reverse transcription) into the host genome by the viral integrase. Su et al (Methods, 2009, volume 47, pages 269-276) disclose site-specific integration of retroviral DNA in human cells using fusion proteins consisting of HIV-1 integrase and the designed polydactyl zinc-finger protein E2C.

[0005] The instant disclosure improves current genome editing technology by allowing one to specifically insert desired nucleic acid (DNA) sequences into the genome at specified locations in the genome. The recombinant engineered integrase with DNA binding ability will bind a given DNA sequence in the genome and recognize a provided DNA sequence having integrase recognition domains (such as the HIV1 (or other retrovirus) att sites) and/or homology arms to insert the given nucleic acid sequence into the genome in a site specific manner. One aspect of the disclosure involves inserting DNA sequences of stop codons (UAA, UAG and/or UGA) just after the transcriptional start site of a gene. This will allow for effective inhibition of gene transcription in the genome of a cell.

SUMMARY

[0006] The current disclosure links DNA targeting technologies, in particular CRISPR/Cas9, with retroviral integrases to form DNA targeting integrases. A gene of interest (GOI) may then be provided with the DNA targeting integrase so that it may be incorporated into the genome in a targeted manner. The GOI may be designed with homology arms to provide another level of specificity to its insertion in the genome.

[0007] The disclosure particularly relates to the use of a variant Cas9 that is inactive for cutting DNA for linking with a retroviral integrase.

[0008] Accordingly, the present invention provides a system for insertion of a donor nucleic acid into genomic DNA, comprising:

1. (a) a fusion protein comprising:
    1. i) a first protein comprising a catalytically inactive Cas9 protein;
    2. ii) a second protein comprising a retroviral integrase, and
    3. iii) a linker linking the first protein to the second protein;
2. (b) a guide RNA (gRNA); and
3. (c) a DNA vector comprising: the donor DNA, a first retroviral long terminal repeat (rLTR) and a second rLTR, wherein the donor DNA is located between the first rLTR and second rLTR. The . The fusion protein may be provided in an expression vector or as a purified protein. The donor DNA (a gene of interest (or DNA sequence of interest) may be provided with or without homology arms to be incorporated into the desired genome. The GOI or DNA sequence of interest may be modified to be recognized by the viral integrase as needed. Other reagents needed for polynucleotide transfection and/or introduction of protein into cells. Assaying for off-target integration of DNA sequences. In one aspect, using a marker sequence engineered into the inserted DNA sequence.

[0009] Disclosed herein are nucleic acid constructs comprising in operable linkage: a) a first polynucleotide sequence encoding an inactive Cas9: b) a second polynucleotide sequence encoding a retroviral integrase; and c) a third polynucleotide sequence encoding a nucleic acid linker; wherein the first polynucleotide sequence comprises a 5' and a 3' end and the second polynucleotide sequence comprises a 5' and a 3' end, and the 3' end of the first polynucleotide is connected to the 5' end of the second polynucleotide by the nucleic acid linker, and the first and second polynucleotide are able to be expressed as a fusion protein in a cell or an organism. In some embodiments, the first polynucleotide sequence comprises any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 27-46, 49, 56, or 68, or a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto. In some embodiments, the inactive Cas9comprises any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 50, or 52, , or a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto. In some embodiments, the second polynucleotide sequence comprises any one of SEQ ID NOS: 15, 17, 19, 21, 47, 55, 62, 70, or 79, or a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto. In some embodiments, the integrase, comprises any one of SEQ ID NOS: 16, 18, 20, 22, 48, 63, 71, or 80, or a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity thereto. Also described herein are organisms comprising the nucleic acid construct. Also described herein is an organism comprising the fusion protein wherein the organism has a modified genome.

[0010] Disclosed herein are organisms comprising: a) a first polynucleotide sequence encoding an inactive Cas9: b) a second polynucleotide sequence encoding an integrase; and c) a third polynucleotide sequence encoding a nucleic acid linker; wherein the first polynucleotide sequence comprises a 5' and a 3' end and the second polynucleotide sequence comprises a 5' and a 3' end, and the 3' end of the first polynucleotide is connected to the 5' end of the second polynucleotide by the nucleic acid linker, and the first and second polynucleotide are able to be expressed as a fusion protein in a cell or an organism.

[0011] Also disclosed herein are fusion proteins, comprising: a) a first protein that is a catalytically inactive Cas9, wherein the first protein is targeted to a target DNA sequence; b) a second protein that is an integrase; and c) a linker linking the first protein to the second protein. In some embodiments, the second protein is an HIV1 integrase or a lentiviral integrase; the linker sequence is one or more amino acids in length; and the first protein is a catalytically inactive Cas9. Also disclosed are instances wherein the linker sequence is 4-8 amino acids in length; the first protein is a TALE protein; or the first protein is a Zinc finger protein. Also disclosed are instances wherein the fusion protein comprises a TALE or a Zinc finger protein, the target DNA sequence is about 16 to about 24 base pairs in length. In some embodiments, the first protein is catalytically inactive Cas9, wherein one or more guide RNAs are used for targeting of a target DNA sequence of from about 16 to about 24 base pairs.

[0012] Also provided herein is an in vitro method of inserting a DNA sequence into genomic DNA, comprising:

1. a) identifying a target sequence in the genomic DNA;
2. b) designing a fusion protein and guide RNA (gRNA) of the present invention to bind to the target sequence in the genomic DNA;
3. c) designing a DNA sequence of interest to incorporate into the genomic DNA; and
4. d) providing the fusion protein, the gRNA, and the DNA sequence of interest to a cell by techniques that allow for entry of the fusion protein, gRNA and DNA sequence of interest into the cell; wherein the DNA sequence of interest becomes integrated at the target sequence in the genomic DNA.

[0013] Also disclosed herein are nucleotide vectors, comprising: a) a first coding sequence for a first protein that is a catalytically inactive Cas9 protein; b) a second coding sequence for a second protein that is an retroviral integrase; c) a DNA sequence between the first and second coding sequences that forms an amino acid linker between the first and second proteins; d) optionally an expressed DNA sequence of interest surrounded by att sites recognized by an integrase, and optionally one or more guide RNAs, wherein the first protein is targeted to a determined DNA sequence, and wherein the first protein is linked to the second protein by the amino acid linker sequence.

[0014] Also provided is an in vitro method of inhibiting gene transcription in a cell, comprising:

1. a) identifying an ATG start codon in a gene;
2. b) designing a fusion protein system with a fusion protein as defined herein and a guide RNA (gRNA) to bind to a target sequence immediately after the ATG start codon of the gene;
3. c) designing a DNA sequence of interest that is one or more consecutive stop codons; and
4. d) providing the fusion protein, the gRNA, and the DNA sequence of interest to a cell by techniques that allow for entry of the fusion protein, gRNA and DNA sequence of interest into the cell; wherein the DNA sequence of interest becomes integrated at the target sequence in the genomic DNA; and wherein transcription of the gene is inhibited.

[0015] In one embodiment, the vector further comprising a reverse transcriptase gene to be expressed in a cell.

[0016] Also disclosed herein are compositions, comprising a purified protein of a DNA binding protein/integrase fusion and an RNA from about 15 to about 100 base pairs in length, wherein the DNA binding protein is a catalytically inactive Cas9 engineered to a targeted DNA sequence in a genome, and wherein the integrase is a HIV integrase, lentiviral integrase, adenoviral integrase, a retroviral integrase, or a MMTV integrase.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, appended claims and accompanying figures where:

FIG. 1 shows a) an exemplary catalytically inactive Cas9/HIV1 integrase fusion protein, b) an exemplary TALE/HIV1 integrase fusion protein, c) an exemplary zinc finger protein/HIV1 integrase fusion protein, and d) an exemplary Cas9/HIV1 integrase fusion protein designed to opposite sides of the DNA at the targeted site. Each of the fusion proteins binds to a specific target sequence of DNA. "ZnFn" is a Zinc finger protein. "Integrase" represents one integrase unit or two integrase units linked, for example, by a short amino acid linker. Cas9 is catalytically inactive.

FIG. 2 shows a DNA plasmid system comprising, a vector comprising a catalytically inactive Cas9/integrase fusion protein, a vector comprising a DNA sequence of interest, and a vector comprising a reverse transcriptase. A guide RNA (gRNA) or RNAs may be provided separately. Another vector can be used to express a gRNA. "1 or 2" refers to one integrase or two integrases linked by, for example, an amino acid linker.

FIG. 3 shows an exemplary DNA plasmid comprising a nucleotide sequence catalytically inactive Cas9/integrase fusion protein, guide RNAs, a DNA (gene) sequence of interest, and a

reverse transcriptase. Viral att sites can be provided to the DNA sequence of interest, allowing for incorporation of the integrase into the cell's genomic DNA. A guide RNA (gRNA) or RNAs may be provided separately. Another vector can be used to express a gRNA. "1 or 2" refers to one integrase or two integrases linked by, for example, an amino acid linker.

FIG. 4 shows a flow diagram. One exemplary method of employing the vectors shown in **FIG. 2** and **FIG. 3** is shown in **FIG. 4,** and is as follows: 1) reverse transcriptase reverse transcribes the DNA sequence of interest with att sites expressed from the vector (alternatively a linear DNA with att sites is used), 2) fusion Cas9/integrase targets site on genomic DNA based on guide RNAs, 3) integrase recognizes att (LTR) sites on DNA sequence of interest and integrates the DNA into the genome at the targeted site, and 4) an assay (e.g. PCR (polymerase chain reaction) is conducted to check for proper insertion of DNA sequence of interest. An assay can be conducted to check for non-specific integration.

FIG. 5 shows Abbie1 Gene Editing Targeting Exon 2 of Nrf2 Using Guide NrF2-sgRNA2 and sgRNA3.

FIG. 6 shows theoretical data generated by Abbie1 gene editing.

FIG. 7 shows Abbie1 Gene Editing Targeting Exon 2 of Nrf2 Using Guide Nrf2-sgRNA 3.

FIG. 8 shows Abbie1 Knock out of Nrf2 in pooled Hek293T Cells.

FIG. 9 shows Abbie1 Knock out of Nrf2 in pooled Hek293T Cells.

FIG. 10 shows Abbie1 Gene Editing Targeting CXCR4 Exon 2.

FIG. 11 shows detection of ABBIE1 protein after isolation and purification from E coli. Coomassie stained gel.

DETAILED DESCRIPTION

[0018] The following detailed description is provided to aid those skilled in the art in practicing the present disclosure.

[0019] As used in this disclosure and the appended claims, the singular forms "a", "an" and "the" include a plural reference unless the context clearly dictates otherwise. As used in this disclosure and the appended claims, the term "or" can be singular or inclusive. For example, A or B, can be A and B.

ENDOGENOUS

[0020] An endogenous nucleic acid, nucleotide, polypeptide, or protein as described herein is defined in relationship to the host organism. An endogenous nucleic acid, nucleotide, polypeptide, or protein is one that naturally occurs in the host organism.

EXOGENOUS

[0021] An exogenous nucleic acid, nucleotide, polypeptide, or protein as described herein is defined in relationship to the host organism. An exogenous nucleic acid, nucleotide, polypeptide, or protein is one that does not naturally occur in the host organism or is a different location in the host organism.

KNOCKOUT

[0022] A gene is considered knocked out when an exogenous nucleic acid is transformed into a host organism (e.g. by random insertion or homologous recombination) resulting in the disruption (e.g. by deletion, insertion) of the gene.

[0023] Upon knocking out a gene, the activity of the corresponding protein can be decreased. For example, by at least 10%, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or 100%, as compared to the activity of the same protein wherein the gene has not been knocked out.

[0024] Upon knockout out of a gene, the transcription of the gene can be decreased, as compared to a gene that has not been knocked out, by at least 20%, by at least 30%, by at least 40%, by at least 50%, by at least 60%, by at least 70%, by at least 80%, by at least 90%, or 100%.

MODIFIED

[0025] A modified organism is an organism that is different than an unmodified organism. For example, a modified organism can comprise a fusion protein of the disclosure that results in a knockout of a targeted gene sequence. A modified organism can have a modified genome.

[0026] A modified nucleic acid sequence or amino acid sequence is different than the unmodified nucleic acid sequence or amino acid sequence. For example, a nucleic acid sequence can have one or more nucleic acids inserted, deleted, or added. For example, an amino acid sequence can have one or more amino acids inserted, deleted, or added.

OPERABLY LINKED

[0027] In some embodiments, a vector comprises a polynucleotide operably linked to one or more control elements, such as a promoter and/or a transcription terminator. A nucleic acid sequence is operably linked when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operatively linked to DNA for a polypeptide if it is expressed as a preprotein which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Operably linked sequences can be contiguous and, in the case of a secretory leader, contiguous and in reading phase.

HOST CELL OR HOST ORGANISM

[0028] A host cell can contain a polynucleotide encoding a polypeptide of the present disclosure. In some embodiments, a host cell is part of a multicellular organism. In other embodiments, a host cell is cultured as a unicellular organism.

[0029] Host organisms can include any suitable host, for example, a microorganism. Microorganisms which are useful for the methods described herein include, for example, bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae),* and plants. The organism can be prokaryotic or eukaryotic. The organism can be unicellular or multicellular.

[0030] The host cell can be prokaryotic. Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of *Escherichia coli, Lactobacillus* sp., *Salmonella* sp., and *Shigella* sp. (for example, as described in Carrier et al. (1992) J. Immunol. 148:1176-1181; U.S. Pat. No. 6,447,784; and Sizemore et al. (1995) Science 270:299-302). Examples of *Salmonella* strains which can be employed in the present disclosure include, but are not limited to, *Salmonella typhi* and *S. typhimurium.* Suitable *Shigella* strains include, but are not limited to, *Shigella flexneri, Shigella sonnei,* and *Shigella disenteriae.* Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, *Pseudomonas pudita, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum,* and *Rhodococcus* sp.

[0031] In some embodiments, the host organism is eukaryotic. Suitable eukaryotic host cells include, but are not limited to, yeast cells, insect cells, plant cells, fungal cells, and algal cells.

POLYNUCLEOTIDES AND POLYPEPTIDES [NUCLEIC ACIDS AND PROTEINS]

[0032] The proteins of the present disclosure can be made by any method known in the art. The protein may be synthesized using either solid-phase peptide synthesis or by classical

solution peptide synthesis also known as liquid-phase peptide synthesis. Using Val-Pro-Pro, Enalapril and Lisinopril as starting templates, several series of peptide analogs such as X-Pro-Pro, X-Ala-Pro, and X-Lys-Pro, wherein X represents any amino acid residue, may be synthesized using solid-phase or liquid-phase peptide synthesis. Methods for carrying out liquid phase synthesis of libraries of peptides and oligonucleotides coupled to a soluble oligomeric support have also been described. Bayer, Ernst and Mutter, Manfred, Nature 237:512-513 (1972); Bayer, Ernst, et al., J. Am. Chem. Soc. 96:7333-7336 (1974); Bonora, Gian Maria, et al., Nucleic Acids Res. 18:3155-3159 (1990). Liquid phase synthetic methods have the advantage over solid phase synthetic methods in that liquid phase synthesis methods do not require a structure present on a first reactant which is suitable for attaching the reactant to the solid phase. Also, liquid phase synthesis methods do not require avoiding chemical conditions which may cleave the bond between the solid phase and the first reactant (or intermediate product). In addition, reactions in a homogeneous solution may give better yields and more complete reactions than those obtained in heterogeneous solid phase/liquid phase systems such as those present in solid phase synthesis.

[0033] In oligomer-supported liquid phase synthesis the growing product is attached to a large soluble polymeric group. The product from each step of the synthesis can then be separated from unreacted reactants based on the large difference in size between the relatively large polymer-attached product and the unreacted reactants. This permits reactions to take place in homogeneous solutions, and eliminates tedious purification steps associated with traditional liquid phase synthesis. Oligomer-supported liquid phase synthesis has also been adapted to automatic liquid phase synthesis of peptides. Bayer, Ernst, et al., Peptides: Chemistry, Structure, Biology, 426-432.

[0034] For solid-phase peptide synthesis, the procedure entails the sequential assembly of the appropriate amino acids into a peptide of a desired sequence while the end of the growing peptide is linked to an insoluble support. Usually, the carboxyl terminus of the peptide is linked to a polymer from which it can be liberated upon treatment with a cleavage reagent. In a common method, an amino acid is bound to a resin particle, and the peptide generated in a stepwise manner by successive additions of protected amino acids to produce a chain of amino acids. Modifications of the technique described by Merrifield are commonly used. See, e.g., Merrifield, J. Am. Chem. Soc. 96: 2989-93

[0035] (1964). In an automated solid-phase method, peptides are synthesized by loading the carboxy-terminal amino acid onto an organic linker (e.g., PAM, 4-oxymethylphenylacetamidomethyl), which is covalently attached to an insoluble polystyrene resin cross-linked with divinyl benzene. The terminal amine may be protected by blocking with t-butyloxycarbonyl. Hydroxyl- and carboxyl-groups are commonly protected by blocking with O-benzyl groups. Synthesis is accomplished in an automated peptide synthesizer, such as that available from Applied Biosystems (Foster City, Calif.). Following synthesis, the product may be removed from the resin. The blocking groups are removed by using hydrofluoric acid or trifluoromethyl sulfonic acid according to established methods. A routine synthesis may produce 0.5 mmole of peptide resin. Following cleavage and purification, a yield of

approximately 60 to 70% is typically produced. Purification of the product peptides is accomplished by, for example, crystallizing the peptide from an organic solvent such as methyl-butyl ether, then dissolving in distilled water, and using dialysis (if the molecular weight of the subject peptide is greater than about 500 daltons) or reverse high pressure liquid chromatography (e.g., using a C^{18} column with 0.1% trifluoroacetic acid and acetonitrile as solvents) if the molecular weight of the peptide is less than 500 daltons. Purified peptide may be lyophilized and stored in a dry state until use. Analysis of the resulting peptides may be accomplished using the common methods of analytical high pressure liquid chromatography (HPLC) and electrospray mass spectrometry (ES-MS).

[0036] In other cases, a protein, for example, a protein is produced by recombinant methods. For production of any of the proteins described herein, host cells transformed with an expression vector containing the polynucleotide encoding such a protein can be used. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell such as a yeast, or the host can be a prokaryotic cell such as a bacterial cell. Introduction of the expression vector into the host cell can be accomplished by a variety of methods including calcium phosphate transfection, DEAE-dextran mediated transfection, polybrene, protoplast fusion, liposomes, direct microinjection into the nuclei, scrape loading, biolistic transformation and electroporation. Large scale production of proteins from recombinant organisms is a well established process practiced on a commercial scale and well within the capabilities of one skilled in the art.

CODON OPTIMIZATION

[0037] One or more codons of an encoding polynucleotide can be "biased" or "optimized" to reflect the codon usage of the host organism. For example, one or more codons of an encoding polynucleotide can be "biased" or "optimized" to reflect chloroplast codon usage or nuclear codon usage. Most amino acids are encoded by two or more different (degenerate) codons, and it is well recognized that various organisms utilize certain codons in preference to others. "Biased" or codon "optimized" can be used interchangeably throughout the specification. Codon bias can be variously skewed in different plants, including, for example, in alga as compared to tobacco. Generally, the codon bias selected reflects codon usage of the plant (or organelle therein) which is being transformed with the nucleic acids of the present disclosure.

[0038] A polynucleotide that is biased for a particular codon usage can be synthesized de novo, or can be genetically modified using routine recombinant DNA techniques, for example, by a site directed mutagenesis method, to change one or more codons such that they are biased for chloroplast codon usage.

PERCENT SEQUENCE IDENTITY

[0039] One example of an algorithm that is suitable for determining percent sequence identity or sequence similarity between nucleic acid or polypeptide sequences is the BLAST algorithm, which is described, e.g., in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (as described, for example, in Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA, 89:10915). In addition to calculating percent sequence identity, the BLAST algorithm also can perform a statistical analysis of the similarity between two sequences (for example, as described in Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA, 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, less than about 0.01, or less than about 0.001.

[0040] The instant disclosure comprises a system comprising: A) A viral integrase covalently linked to a catalytically inactive Cas9 protein that is, for example, inactive for DNA cutting ability. Also disclosed is the viral integrase (or a bacterial or phage recombinase) covalently linked to a TALE protein or zinc finger proteins where these proteins are designed to target a specific sequence of DNA in a genome.

[0041] This may be provided in an expression vector or as a purified protein. B) A gene of interest (or DNA sequence of interest) with or without homology arms to be incorporated into the desired genome. The GOI or DNA sequence of interest may be modified to be recognized by the viral integrase as needed. For example, the viral att sites can be added to the ends of the DNA sequence. C) Other reagents needed for polynucleotide transfection and/or introduction of protein into cells.

NUCLEIC ACID

[0042] The terms "polynucleotide", "nucleotide", "nucleotide sequence", "nucleic acid" and "oligonucleotide" are used interchangeably in this disclosure. 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.

GUIDE RNA

[0043] In aspects of the disclosure 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 (12-30 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)".

WILD TYPE

[0044] 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.

VARIANT

[0045] As used herein the terms "variant" or "mutant" should be taken to mean the exhibition of qualities that have a pattern that deviates from what occurs in nature. In relation to the genes, these terms indicate a number of changes in a gene that make it different from the wild-type gene including single nucleotide polymorphisms (SNPs), insertions, deletions, gene shifts among others.

ENGINEERED

[0046] The terms "non-naturally occurring" or "engineered" are used interchangeably and indicate the involvement of man-made technology. 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.

COMPLEMENTARY

[0047] "Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick 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%, 70%, 75%, 80%, 85%, 90%, 95%. 97%, 98%, 99%, or 100%, or percentages in between 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.

AMINO ACIDS

[0048]

Full Name, Three-Letter Code, One-Letter Code

Aspartic Acid Asp D

Glutamic Acid Glu E

Lysine Lys K

Arginine Arg R

Histidine His H

Tyrosine Tyr Y

Cysteine Cys C

Asparagine Asn N

Glutamine Gln Q

Serine Ser S

Threonine Thr T

Glycine Gly G

Alanine Ala A

Valine Val V

Leucine Leu L

Isoleucine Ile I

Methionine Met M

Proline Pro P

Phenylalanine Phe F

Tryptophan Trp W

[0049] The expression "amino acid" as used herein is meant to include both natural and synthetic amino acids, and both D and L amino acids. "Standard amino acid" means any of the twenty standard L-amino acids commonly found in naturally occurring proteins/peptides. "Non-standard amino acid residue" means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or derived from a natural source. As used herein, "synthetic amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the peptides of the present disclosure, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups which can change the peptide's circulating half-life without adversely affecting their activity. Additionally, a disulfide link may be present or absent in the peptides.

[0050] Amino acids may be classified into seven groups on the basis of the side chain R: (1) aliphatic side chains; (2) side chains containing a hydroxyl (OH) group; (3) side chains containing sulfur atoms; (4) side chains containing an acidic or amide group; (5) side chains containing a basic group; (6) side chains containing an aromatic ring; and (7) proline, an imino acid in which the side chain is fused to the amino group.

[0051] As used herein, the term "conservative amino acid substitution" is defined herein as exchanges within one of the following five groups:

1. I. Small aliphatic, nonpolar or slightly polar residues:
   Ala, Ser, Thr, Pro, Gly;
2. II. Polar, negatively charged residues and their amides:
   Asp, Asn, Glu, Gin;
3. III. Polar, positively charged residues:
   His, Arg, Lys;
4. IV. Large, aliphatic, nonpolar residues:
   Met Leu, He, Val, Cys (Ile; autocorrect is not literate)

5. V. Large, aromatic residues:
   Phe, Tyr, Tip (Trp, likewise)

[0052] The present disclosure utilizes, unless otherwise provided, 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)).

VECTORS

[0053] Gene expression vectors (DNA-based or viral) will be used to express the fusion integrases in cells or tissues as well as to provide the DNA sequence (or gene) of interest with the appropriate sites needed for the integrase or recombinase to integrate that DNA (or gene) into the genome of the host species or cell. A number of gene expression vectors are known in the art. Vectors will be use for the gene of interest (or DNA sequence of interest). Vectors may be cut with a number of restriction enzymes known in the art.

CRISPR/CAS9

[0054] CRISPR/Cas9 is described in US Patent 8697359, US 8889356 and Ran et al (Nature Protocols, 2013, volume 8, pages 2281-2308). Cas9 protein utilizes RNA guides in order to bind specific sequences of DNA in a genome. The RNA guides (guide RNAs) may be designed to be from 10 to 40, from 12 to 35, from 15 to 30, or for example, from 18 to 22, or 20 nucleotides in length. See Hsu et al, Nature Biotechnology, September 2013, volume 31, pages 827-832, which uses Cas9 from Streptococcus pyogenes. Another key Cas9 is from Staphylococcus Aureus (a smaller Cas9 than that of S pyogenes). The Cas9 protein utilizes guide RNAs to bind specific regions of a DNA sequence.

[0055] The present invention uses a catalytically inactive Cas9 protein as part of a fusion protein. A catalytically inactive form of Cas9 is described in Guilinger et al, Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification, Nature Biotechnology, April 25, 2014, volume 32, pages 577-582. Guilinger et al attached the catalytically inactive Cas9 to a Fok1 enzyme to achieve greater specificity in making cuts in genomic DNA. This catalytically inactive Cas9 allows for Cas9 to use RNA guides for binding of genomic DNA, while not being able to cut the DNA.

[0056] Cas9 is also available in its natural wt form, and also a human optimized codon form for better expression of Cas9 constructs in cells. (see Mali et al, Science, 2013, volume 339, pages 823-826). Codon optimization of Cas9 may be conducted dependent on the species for its expression. Depending on whether one produces a protein form of the Integrase/Cas9 fusion protein (also known as ABBIE1) or a nucleotide expression vector form, the optimized or non-optimized (wt) form may be used.

[0057] RNA guides toward a specific DNA sequence can be designed by various computer-based tools.

CRISPR/CPF1

[0058] Cpfl is another protein, which uses a guide RNA in order to bind a specific sequence in genomic DNA. Cpfl also cuts DNA making a staggered cut. Cpfl may be made to be catalytically inactive for cutting ability.

OTHER CRISPR PROTEINS

[0059] These are proteins that utilize a guide RNA to target a specific DNA sequence and whether they have the ability to cut DNA or not. Some of these proteins may naturally have other enzymatic/catalytic functions.

TALEN

[0060] Also disclosed are TALENs. Transcription Activator-Like Effector Nucleases (TALENs) are fusion proteins with restriction enzymes generated by fusing the TAL effector DNA binding domain to a DNA cleavage domain. These reagents enable efficient, programmable, and specific DNA cleavage and represent powerful tools for genome editing in situ. Transcription activator-like effectors (TALEs) can be quickly engineered to bind practically any DNA sequence. The term TALEN, as used herein, is broad and includes a monomeric TALEN that can cleave double stranded DNA without assistance from another TALEN. The term TALEN is also used to refer to one or both members of a pair of TALENs that are engineered to work together to cleave DNA at the same site. TALENs that work together may be referred to as a left-TALEN and a right-TALEN, which references the handedness of DNA. See US 8, 440,432.

[0061] TAL effectors are proteins secreted by Xanthomonas bacteria. The DNA binding domain contains a highly conserved 33-34 amino acid sequence with the exception of the 12th and 13th amino acids. These two locations are highly variable (Repeat Variable Diresidues (RVD)) and show a strong correlation with specific nucleotide recognition. This simple

relationship between amino acid sequence and DNA recognition has allowed for the engineering of specific DNA binding domains by selecting a combination of repeat segments containing the appropriate RVDs.

[0062] The integrase can be used to construct hybrid integrase or recombinase that are active in a yeast or cell assay. These reagents are also active in plant cells and in animal cells. TALEN studies used the wild-type FokI cleavage domain, but some subsequent TALEN studies also used FokI cleavage domain variants with mutations designed to improve cleavage specificity and cleavage activity. Both the number of amino acid residues between the TALEN DNA binding domain and the integrase or recombinase domain and the number of bases between the two individual TALEN binding sites are parameters for achieving high levels of activity. The number of amino acid residues between the TALEN DNA binding domain and the integrase or recombinase domain may be modified by introduction of a spacer (distinct from the spacer sequence) between the plurality of TAL effector repeat sequences and the integrase or recombinase domain. The spacer sequence may be 6 to 102 or 9 to 30 nucleotides or 15 to 21 nucleotides. These spacers will usually not provide other activity to the hybrid protein besides providing a link between the DNA targeting protein (Cas9, TALE or zinc finger protein) and the integrase or recombinase. The amino acids for the spacers and for other uses in the instant disclosure are

[0063] The relationship between amino acid sequence and DNA recognition of the TALEN binding domain allows for designable proteins. In this case artificial gene synthesis is problematic because of improper annealing of the repetitive sequence found in the TALE binding domain. One solution to this is to use a publicly available software program named DNAWorks to find oligonucleotides suitable for assembly in a two step PCR; oligonucleotide assembly followed by whole gene amplification. A number of modular assembly methods for generating engineered TALE constructs have also been reported in the art.

[0064] Once the TALEN genes have been assembled together they are inserted into plasmids; the plasmids are then used to transfect the target cell where the gene products are expressed and enter the nucleus to access the genome. TALENs can be used to edit genomes by inducing double-strand breaks (DSB), which cells respond to with DNA repair, however, the instant disclosure seeks to use the power of viral integrases to insert DNA sequences of interest into targeted sites in the genome. See disclosure of WO 2014134412 and US Patent 8748134.

ZINC FINGER PROTEINS

[0065] Also disclosed are Zinc finger proteins. Zinc finger proteins for binding DNA and their design are described in US 7928195, US 2009/0111188, and US 7951925. Zinc finger proteins utilize a number of linked zinc finger domains in a specified order to bind to a specific sequence of DNA. Zinc finger protein endonucleases have been well-established.

[0066] Zinc finger proteins (ZFPs) are proteins that can bind to DNA in a sequence-specific manner. Zinc fingers were first identified in the transcription factor TFIIIA from the oocytes of the African clawed toad, Xenopus laevis. A single zinc finger domain of this class of ZFPs is about 30 amino acids in length, and several structural studies have demonstrated that it contains a beta turn (containing two conserved cysteine residues) and an alpha helix (containing two conserved histidine residues), which are held in a particular conformation through coordination of a zinc atom by the two cysteines and the two histidines. This class of ZFPs is also known as C2H2 ZFPs. Additional classes of ZFPs have also been suggested. See, e.g., Jiang et al. (1996) J. Biol. Chem. 271:10723-10730 for a discussion of Cys-Cys-His-Cys (C3H) ZFPs. To date, over 10,000 zinc finger sequences have been identified in several thousand known or putative transcription factors. Zinc finger domains are involved not only in DNA recognition, but also in RNA binding and in protein-protein binding. Current estimates are that this class of molecules will constitute about 2% of all human genes.

[0067] Many zinc finger proteins have conserved cysteine and histidine residues that tetrahedrally-coordinate the single zinc atom in each finger domain. In particular, most ZFPs are characterized by finger components of the general sequence: -Cys-(X)2-4-Cys-(X)12-His-(X)3-5-His- (SEQ ID NO:49, in which X represents any amino acid (the C2H2 ZFPs). The zinc-coordinating sequences of this most widely represented class contain two cysteines and two histidines with particular spacings. The folded structure of each finger contains an antiparallel β-turn, a finger tip region and a short amphipathic α-helix. The metal coordinating ligands bind to the zinc ion and, in the case of zif268-type zinc fingers, the short amphipathic α-helix binds in the major groove of DNA. In addition, the structure of the zinc finger is stabilized by certain conserved hydrophobic amino acid residues (e.g., the residue directly preceding the first conserved Cys and the residue at position +4 of the helical segment of the finger) and by zinc coordination through the conserved cysteine and histidine residues.

OTHER DNA BINDING PROTEINS THAT MAY BIND SPECIFIC TARGET SEQUENCES IN GENOMIC DNA

[0068] The proteins disclosed include those unrelated to the zinc finger proteins, TALEN and CRISPR proteins that may bind to specific sequences in genomic DNA of various organisms. These may include transcription factors, transcriptional repressors, meganucleases, endonuclease DNA binding domains and others.

INTEGRASES

[0069] Integrases and endonuclease fusion proteins thereof are described in US 2009/0011509. Integrases introduced are lentiviral integrase and HIV1 (human immunodeficiency virus 1) integrase. The instant disclosure fuses a catalytically inactive Cas9 to an integrase via a linker to target the integrase to a specific region of DNA in the genome

that is chosen by the user.

[0070] The HIV-1 integrase, like other retroviral integrases, is able to recognize special features at the ends of the viral DNA located in the U3 and U5 regions of the long terminal repeats (LTRs) (Brown, 1997). The LTR termini are the only viral sequences thought to be required in cis for recognition by the integration machinery of retroviruses. Short imperfect inverted repeats are present at the outer edges of the LTRs in both murine and avian retroviruses (reviewed by Reicin et al., 1995). Along with the subterminal CA located at the outermost positions 3 and 4 in retroviral DNA ends (positions 1 and 2 being the 3' end processed nucleotides, these sequences are both necessary and sufficient for correct proviral integration in vitro and in vivo. Sequences internal to the CA dinucleotide appear to be important for optimal integrase activity (Brin & Leis, 2002a; Brin & Leis, 2002b; Brown, 1997). The terminal 15 bp of the HIV-1 LTRs have been shown to be crucial for correct 3' end processing and strand transfer reactions in vitro (Reicin et al., 1995; Brown, 1997). Longer substrates are used more efficiently than shorter ones by HIV-1 IN which indicates that binding interactions extend at least 14-21 bp inward from the viral DNA end. Brin and Leis (2002a) analysed the specific features of the HIV-1 LTRs and concluded that both the U3 and U5 LTR recognition sequences are required for IN-catalysed concerted DNA integration, even though the U5 LTRs are more efficient substrates for IN processing in vitro (Bushman & Craigie, 1991; Sherman et al., 1992). The positions 17-20 of the INrecognition sequences are needed for a concerted DNA integration mechanism, but the HIV-1 IN tolerates considerable variation in both the U3 and U5 termini extending from the invariant subterminal CA dinucleotide (Brin & Leis, 2002b). The instant disclosure includes a DNA vector that contains viral (retroviral or HIV) LTR regions at the 5' and 3' ends of a location to house the DNA sequence or gene of interest to be integrated into the genome. The LTR regions do not have to be the full length LTRs as long as they function to interact with the integrase for proper integration. The LTR regions may be modified to contain detectable (e.g. fluorescent), PCR detection, or selectable markers (e.g. antibiotic resistance). The vector is designed to be cut and linearized so that the LTR regions are at the 5' and 3' ends of the DNA fragment (via designed restriction sites to restriction endonuclease).

[0071] Integrases consist of three domains connected by flexible linkers. These domains are an N-terminal HH-CC zinc-binding domain, a catalytic core domain and a C-terminal DNA binding domain (Lodi et al, Biochemistry, 1995, volume 34, pages 9826-9833). In some aspects of the disclosure the integrase bound to the Cas9 (or other DNA binding molecule) will not have the C-terminal binding domain. In one aspect of the disclosure, two different fusion proteins will be produced where one has catalytically inactive Cas9 fused with the N-terminal zinc binding domain of an integrase and the other has catalytically inactive Cas9 fused with the catalytic core domain of the integrase. The two different fusion proteins will be designed to bind to opposite strands of the genomic DNA as seen with TALE-Fok1 or Zinc finger-Fok1 systems. In this manner, when the N-terminal domain and the catalytic core come in contact, at the site on the genomic DNA, it will exhibit integrase activity. As full activity of integrase has also been observed to involve tetramers of integrase, fusion proteins may be designed with 1, 2, 3, 4 integrase proteins linked by flexible linkers that may be 1 to 20 amino acids in length or 4-12

amino acids in length.

RECOMBINASES

[0072] Also disclosed are recombinases. Recombinases including Cre, Flp, R, Dre, Kw, and Gin recombinase are described in US 8816153 and US 2004/0003420. Recombinases such as Cre recombinase use LoxP sites in order to excise a sequence from the genome. Recombinases can be modified to become constitutively active for their recombination activity and also become less site specific. Thus, it is possible to target such constitutively active recombinase proteins with no sequence specificity to specific sequences of DNA in a genome by incorporating them into fusion proteins of the instant disclosure. In this manner, the CRISPR/Cas9 specifies the DNA sequence where the recombinase will contribute its recombination activity. Such recombinase proteins may be wild-type, constitutively active or dead for recombinase activity. A Cas9-recombinase such as Cas9-Gin or Cas9-Cre may be produced by use of a linker sequence or by direct fusion.

NUCLEAR LOCALIZATION SIGNAL SEQUENCE (NLS) FOR FUSION PROTEINS

[0073] The signal peptide domain (also referred to as "NLS") is, for example, derived from yeast GAL4, SKI3, L29 or histone H2B proteins, polyoma virus large T protein, VP1 or VP2 capsid protein, SV40 VP1 or VP2 capsid protein, Adenovirus E1a or DBP protein, influenza virus NS1 protein, hepatitis virus core antigen or the mammalian lamin, c-myc, max, c-myb, p53, c-erbA, jun, Tax, steroid receptor or Mx proteins (see Boulikas, Crit. Rev. Eucar. Gene Expression, 3, 193-227 (1993)), simian virus 40 ("SV40") T-antigen (Kalderon et. al, Cell, 39, 499-509 (1984)) or other proteins with known nuclear localization. The NLS is, for example, derived from the SV40 T-antigen, but may be other NLS sequences known in the art. Tandem NLS sequences may be used.

LINKER REGIONS

[0074] The various linkers used between fusion proteins/peptides being synthesized will be composed of amino acids. At the DNA level, these are represented by 3 base pair (bp) codons as known in the genetic code. Linkers may be from 1 to 1000 amino acids in length and any integer in between. For example, linkers are from 1 to 200 amino acids in length or linkers are from 1 to 20 amino acids in length.

EXPRESSION VECTORS

[0075] Many nucleic acids may be introduced into cells to lead to expression of a gene. As

used herein, the term nucleic acid includes DNA, RNA, and nucleic acid analogs, and nucleic acids that are double-stranded or single-stranded (i.e., a sense or an antisense single strand). Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety include deoxyuridine for deoxythymidine, and 5-methyl-2'-deoxycytidine and 5- bromo-2'-doxycytidine for deoxycytidine. Modifications of the sugar moiety include modification of the 2' hydroxyl of the ribose sugar to form 2'-0-methyl or 2'-0-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. See, Summerton and Weller (1997) Antisense Nucleic Acid Drug Dev. 7(3): 187; and Hyrup et al. (1996) Bioorgan. Med. Chem. 4:5. In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone. Nucleic acid sequences can be operably linked to a regulatory region such as a promoter. Regulatory regions can be from any species. As used herein, operably linked refers to positioning of a regulatory region relative to a nucleic acid sequence in such a way as to permit or facilitate transcription of the target nucleic acid. Any type of promoter can be operably linked to a nucleic acid sequence. Examples of promoters include, without limitation, tissue-specific promoters, constitutive promoters, and promoters responsive or unresponsive to a particular stimulus (e.g., inducible promoters).

[0076] Additional regions that may be useful in nucleic acid constructs, include, but are not limited to, polyadenylation sequences, translation control sequences (e.g., an internal ribosome entry segment, IRES), enhancers, inducible elements, or introns. Such regulatory regions may not be necessary, although they may increase expression by affecting transcription, stability of the mRNA, translational efficiency, or the like. Such regulatory regions can be included in a nucleic acid construct as desired to obtain optimal expression of the nucleic acids in the cell(s). Sufficient expression can sometimes be obtained without such additional elements.

[0077] A nucleic acid construct may be used that encodes signal peptides or selectable markers. Signaling (marker) peptides can be used such that an encoded polypeptide is directed to a particular cellular location (e.g., the cell surface). Non-limiting examples of such selectable markers include puromycin, ganciclovir, adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo, G418, APH), dihydrofolate reductase (DHFR), hygromycin-B-phosphtransferase, thymidine kinase (TK), and xanthin-guanine phosphoribosyltransferase (XGPRT). These markers are useful for selecting stable transformants in culture. Other selectable markers include fluorescent polypeptides, such as green fluorescent protein, red fluorescent, or yellow fluorescent protein.

[0078] Nucleic acid constructs can be introduced into cells of any type using a variety of biological techniques known in the art. Non-limiting examples of these techniques would include the use of transposon systems, recombinant viruses that can infect cells, or liposomes

or other non-viral methods such as electroporation, microinjection, or calcium phosphate precipitation, that are capable of delivering nucleic acids to cells. A system called Nucleofection.TM. may also be used.

[0079] Nucleic acids can be incorporated into vectors. A vector is a broad term that includes any specific DNA segment that is designed to move from a carrier into a target DNA. A vector may be referred to as an expression vector, or a vector system, which is a set of components needed to bring about DNA insertion into a genome or other targeted DNA sequence such as an episome, plasmid, or even virus/phage DNA segment. Vectors most often contain one or more expression cassettes that comprise one or more expression control sequences, wherein an expression control sequence is a DNA sequence that controls and regulates the transcription and/or translation of another DNA sequence or mRNA, respectively.

[0080] Many different types of vectors are known in the art. For example, plasmids and viral vectors, including retroviral vectors, are known. Mammalian expression plasmids typically have an origin of replication, a suitable promoter and optional enhancer, and also any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non- transcribed sequences. Such vectors include plasmids (which may also be a carrier of another type of vector), adenovirus, adeno-associated virus (AAV), lentivirus (e.g., modified HIV-1, SIV or FIV), retrovirus (e.g., ASV, ALV or MoMLV), and transposons (P-elements, Tol-2, Frog Prince, piggyBac or others).

[0081] Bacterial and viral genes and proteins for use in the disclosure are listed below in the section entitled "SEQUENCES OF THE DISCLOSURE". Other viral integrases, for example, those from mouse mammary tumor virus (MMTV) and adenovirus can also be used in the methods and compositions disclosed herein.

[0082] A pooled population of edited cells are considered a mixture of cells that have received a gene edit and cells that have not.

EXEMPLARY ABBIE1 IN VITRO ASSAY

[0083]

1. 1) Incubate ABBIE1 protein with guide RNA;
2. 2) Incubate ABBIE1/guide RNA with donor DNA having partial LTRs to form pre-initiation complex;
3. 3) Incubate pre-initiation complex with plasmid containing gene to be edited (e.g. CXCR4); and
4. 4) PCR and DNA sequencing confirmations for donor DNA integration.

[0084] Cas9protocols are described in, for example, Gagnon et al., 2014, http://labs.mcb.harvard.edu/schier/VertEmbryo/Cas9_Protocols.pdf.

[0085] Assays for integrase activity are described in, for example, Merkel et al., Methods, 2009, volume 47, pages 243-248.

EXAMPLES

[0086] The following examples are intended to provide illustrations of the application of the present disclosure. The following examples are not intended to completely define or otherwise limit the scope of the disclosure.

EXAMPLE 1: DNA VECTORS FOR EXPRESSION CAS9-INTEGRASE FUSION PROTEINS

[0087] The DNA sequence of catalytically inactive Cas9 is incorporated into an expression vector with a 12, 15, 18, 21, 24, 27 or 30 bp spacer (codes for 4, 5, 6, 7, 8, 9 or 10 amino acids as the linker between the Cas9 and the integrase) and the HIV1 integrase. In other experiments, recombinases of bacterial or phage origin are used rather than integrases. These include Hin recombinase (SEQ ID NO: 25) and Cre recombinase (SEQ ID NO: 26) with or without mutations that allow them to recombine DNA at any other sites. A His or cMyc tag (or other sequence useful for protein purification) may be included to isolate the fusion protein. The expression vector uses a promoter that will be activated in the cells that will be provided with the vector. The CMV (cytomegalovirus promoter) is commonly used for expression vectors for mammalian cells. The U6 promoter is also commonly used. A T7 promoter may be used for in vitro transcription in certain embodiments.

EXAMPLE 2: DNA VECTOR FOR EXPRESSION OF THE DNA SEQUENCE OF INTEREST (GENE OF INTEREST)

[0088] The DNA sequence of interest will be inserted into the appropriate expression vector and sites will be appropriately added to the DNA sequence of interest so the HIV1 integrase will recognize the sequences for integration into the genome. These sites are termed att sites (U5 and U3 att sites) (see Masuda et al, Journal of Virology, 1998, volume 72, pages 8396-8402). Homology arms for the target site in the genome can be included in regions flanking the 5' and 3' ends of the DNA (gene) sequence of interest (see Ishii et al, PLOS ONE, September 24, 2014, DOI: 10.1371/journal.pone.0108236). When using a recombinase, the integrase recognition sites may not be included. Markers, such as drug resistance markers (e.g. blasticidin or puromycin), will be included in order to check for insertion of the DNA sequence of interest and to help assay for random insertions in the genome. These resistance markers can be engineered in such a way to remove them from the targeted genome landing pad For

example flanking the puromycin resistance gene with a LoxP sites and introducing exogenously expressed CRE would remove the internal sequence leaving a scar containing a LoxP site.

EXAMPLE 3: DNA VECTOR FOR REVERSE TRANSCRIPTASE EXPRESSION

[0089] A reverse transcriptase may also be co-expressed in such systems as the designed DNA sequence (Gene) of interest in the vector will become expressed as RNA and will have to be converted back to DNA for integration by the integrase enzyme. The reverse transcriptase may be viral in origin (e.g. a retrovirus such as HIV1). This may be incorporated within the same vector as the DNA sequence of interest.

EXAMPLE 4: CO-EXPRESSION OF DNA TARGETING-INTEGRASES (OR RECOMBINASES) WITH DNA SEQUENCE OF INTEREST

[0090] Cells were electroporated for the vectors described above along with the Cas9 RNA guides required for the target site in the genome. In some experiments, vectors were created that expressed all of the components (fusion Cas9/integrase (or recombinase), the Cas9 RNA guides, and the DNA sequence of interest with integrase recognition sites and with or without homology arms). A reverse transcriptase may also be co-expressed in such systems as the designed DNA sequence (Gene) of interest in the vector will become expressed as RNA and will have to be converted back to DNA for integration by the integrase enzyme. The reverse transcriptase may be viral in origin (e.g. a retrovirus such as HIV1). In other experiments, the DNA sequence of interest in linearized before introduction to the cell. The Cas9 RNA guide sequences and DNA sequence of interest had to be designed and inserted into the vector before use by standard molecular biology protocols.

EXAMPLE 5: TEST EXPERIMENTS AND ASSAYING FOR OFF-TARGET INSERTIONS

[0091] Cells missing expression of a particular gene, such as mouse embryonic fibroblasts from a knockout mouse model or cells genetically engineered to be knockouts for a given gene, are transfected or electroporated with the above vectors where the gene of interest is included. Chimeric primer sets designed to cover the inserted gene as well as flanking genomic sequence will be used to screen initial pools of edited cells. Limited dilution cloning (LDC) and or FACS analysis is then performed to ensure monoclonality. Next generation sequencing (NGS) or single nucleotide polymorphism (SNP) analysis is performed as a final quality control step to ensure isolated clones are homogenous for the designed edit. Other mechanisms for screening can include but are not limited to qRT-PCR and western blotting with appropriate antibodies. If the protein is associated with a certain phenotype of the cells, the cells may be examined for rescue of that phenotype. The genomes of the cells are assayed for the

specificity of the DNA insertion and to find the relative number of off-target insertions, if any.

EXAMPLE 6: CAS9 LINKED INTEGRASE PROTEIN EXPRESSION AND ISOLATION

[0092] Vectors designed for gene expression in E coli or insect cells will be incorporated into E coli or insect cells and allowed to express for a given period of time. Several designs will be utilized to generate Cas9 (or inactive Cas9) linked integrase protein. The vectors will also incorporate a tag that is not limited to a His or cMyc tag for eventual isolation of the protein with high purity and yield. Preparation of the chimeric protein will include but are not limited to standard chromatography techniques. The protein may also be designed with one or more NLS (nuclear localization signal sequence) and/or a TAT sequence. The nuclear localization signal allows the protein to enter the nucleus. The TAT sequence allows for easier entry of a protein into a cell (it is a cell-penetrating peptide). Other cell penetrating peptides in the art may be considered. After sufficient time for expression has occurred, protein lysate will be collected from the cells and purified in the appropriate column depending on the tag used. The purified protein will then be placed in the appropriate buffering solution and stored at either -20 or -80 degrees C.

EXAMPLE 7: USING CAS9-INTEGRASE TO INCORPORATE STOP CODONS JUST UPSTREAM OF TRANSCRIPTION START SITE

[0093] The disclosure includes a method to create a knockout cell line or organism. The above system is used with the DNA sequence of interest being 1, 3, 6, 10, 15 or 20 consecutive stop codons to be placed just after the ATG start site for the target gene. This will create an effective gene knockout as transcription/translation will be stopped when reaching the immediate stop codon after the ATG start site. Additional stop codons will help prevent possible run through of the transcriptase (if transcriptase by-passes the first stop codon).

EXAMPLE 8: USING ABBIE1 (OR OTHER VARIATIONS HAVING OTHER SPECIFIC DNA BINDING DOMAINS) AS A PURIFIED PROTEIN TO EDIT THE GENOMES OF CELLS

[0094] Incubate Abbie1 isolated protein (other specific DNA sequence binding protein linked to retroviral integrase) with insertable/integratable DNA having viral LTR regions in a suitable buffer. (for formation of tetramer or other multimer depending on the instance). Alternatively, a premade composition of isolated Abbie1 protein with guide RNA may be combined with the insertable DNA sequence. Include guide RNA and incubate to incorporate guide RNA. Transfect or electroporate (or other technique of providing protein to cells) Abbie1/DNA preparation into cells. Allow time for genome/DNA editing to take place. Check for insertion of designed insertable DNA sequence into the specific site of the genomic DNA of the cell. Check for non-specific insertions by PCR and DNA sequencing.

[0095] As currently planned, the bacterial expression vector will be the pMAL-c5e, which is a discontinued product from NEB and one of the in-house cloning choices for Genscript. Codon-optimized Spy Cas9 is cloned with the his-tag and the TEV protease cleavage site in frame with the maltose-binding protein (MBP) tag. The ORF is under the inducible Tac promoter, and the vector also codes for the lac repressor (LacI) for tighter regulation. MBP will be used only as a stabilization tag and not a purification tag, for the amylose resin is quite expensive. The soluble expressed material will be purified over the Ni-affinity chromatography, then Cas9 is released by the TEV protease from MBP, purified by cation exchange chromatography, and polished by gel filtration.

EXAMPLE 9: DESIGN OF CONSTRUCTS FOR FUSION PROTEINS

[0096] Design sequence specific Zinc finger domain, TALE, or guide RNA for CRISPR based approach toward a target DNA sequence. Use on-line design software of choice.

[0097] Produce DNA construct with coding sequences for integrase, transposase or recombinase; a suitable amino acid linker; the appropriate zinc finger, TALE or CRISPR protein (e.g. Cas9, Cpfl); and a nuclear localization signal (or mitochondrial localization signal) to form the site specific fusion integrase protein. These are envisioned in multiple arrangements. A suitable tag may be included for protein isolation and purification if desired (e.g. maltose binding protein (MBP) or His tag).

[0098] DNA construct may utilize a mammalian cell promoter or a bacterial promoter common in the art (e.g. CMV, T7, etc.)

[0099] One may produce a recombinant fusion protein with E coli as the source. Isolate the protein by standard means in the art (e.g. MBP columns, nickel-sepharose columns, etc.).

[0100] Assemble the Donor-RNP complex (duplex the RNA oligos and mix with fusion protein of the invention (when fusion protein has an endonuclease inactive CRISPR related protein for its DNA binding ability, e.g. ABBIE1) - these steps of forming RNP are not necessary for Zinc finger domains and TALE.

1. 1. Mix Donor DNA with appropriate LTR domains and insertable sequence, and fusion protein and incubate for 10 minutes. (alternatively add Donor DNA after the RNP complex formation)
2. 2. Resuspend each RNA oligo (crRNA and tracrRNA) in Nuclease-Free IDTE Buffer. For example, use a final concentration of 100 µM.
3. 3. Mix the two RNA oligos in equimolar concentrations in a sterile microcentrifuge tube. For example, create a final duplex concentration of 3 µM using the following table: Component Amount 100 µM crRNA 3 µL 100 µM tracrRNA 3 µL Nuclease-Free Duplex Buffer 94 µL Final volume 100 µL

4. 4. Heat at 95° C for 5 min.
5. 5. Remove from heat and allow to cool to room temperature (15-25°C) on your bench top.
6. 6. If needed, dilute duplexed RNA to a working concentration (for example, 3 µM) in Nuclease-Free Duplex Buffer.
7. 7. Dilute fusion protein to a working concentration (for example, 5 µM) in Working Buffer (20 mM HEPES, 150 mM KCl, 5% Glycerol, 1mM DTT, pH 7.5).
8. 8. For each transfection, combine 1.5 pmol of duplexed RNA oligos (Step A5) with 1.5 pmol of fusion protein (Step A6) in Opti-MEM Media to a final volume of 12.5 µL.
9. 9. Incubate at room temperature for 5 min to assemble the RNP complexes.

EXAMPLE 10: REVERSE TRANSFECT GRNA-FUSION PROTEIN IN A 96-WELL PLATE

[0101]

1. 1. Incubate the following at room temperature for 20 min to form transfection complexes: Component Amount RNP (Step A8) 12.5 µL Lipofectamine® RNAiMAX Transfection Reagent 1.2 µL Opti-MEM® Media 11.3 µL Total volume 25.0 µL
2. 2. During incubation (Step B1), dilute cultured cells to 400,000 cells/mL using complete media without antibiotics.
3. 3. When incubation is complete, add 25 µL of transfection complexes (from Step B1) to a 96-well tissue culture plate.
4. 4. Add 125 µL of diluted cells (from Step B2) to the 96-well tissure culture plate (50,000 cells/well; final concentration of RNP will be 10 nM).
5. 5. Incubate the plate containing the transfection complexes and cells in a tissure culture incubator (37°C, 5% CO2) for 48 hr. To detect on-target mutations, use PCR with appropriate primers (primers within donor sequence and primers surrounding the target insertion site).

EXAMPLE 11: PROTOCOL FOR TESTING THE SPECIFICITY OF CRISPR/CAS9

[0102] Produce dCas9 (DNA cutting inactive Cas9) linked to biotin (dCas9-biotin). Cas9 (s pyogenes, s aureus, etc.). Biotinylation methods are described below.

[0103] Biotinylation method #1: engineer the avi-tag (~15 residues) at the N- or C-terminus, express and purify as the WT (un-tagged) protein. Use the E. coli biotin ligase (BirA) and biotin to biotinylate the avi-tagged Cas9. We use this scheme to biotinylate chemokines. I believe the IP on the avi-tag technology expired a few years ago.

[0104] Biotinylation method #2.1: biotin functionalized with succinimidyl-ester can be incorporated at surface-exposed lysines residues (no enzymatic reaction required). For proteins as big as Cas9, this can be a viable option.

[0105] Biotinylation method #2.2: along the same line, biotin-maleimide is commercially available, and they can be conjugated at surface-exposed cysteines (no enzyme).

[0106] Testing will be accomplished to characterize the biotinylated Cas9 does in terms of DNA-binding and cleavage.

[0107] Streptavidin-coated 96-well plates are commercially available, but may also be produced in-house.

[0108] Bind dCas9-biotin to plastic plates (96-well, 24-well, 384-well, etc.).

[0109] Provide designed guide RNAs to each well. Allow time for guide RNAs to interact with Cas9 protein.

[0110] Provide genomic DNA to each well or DNA with targeted sequence. Allow time for Cas9 binding to DNA.

[0111] Wash wells with appropriate buffer.

[0112] Provide an adapter (DNA oligomer). Allow time to bind.

[0113] Restriction-digest the genomic DNA to make it more tractable and easier to ligate the adapter.

[0114] Wash wells.

[0115] Perform DNA sequencing to identify sites of binding (on target vs. off target).

EXAMPLE 12: NRF2 EDITING VIA ABBIE 1

[0116] FIG. 5 shows Abbie1 Gene Editing Targeting Exon 2 of Nrf2 Using Guide NrF2-sgRNA2 and sgRNA3. PCR screen against exon 2 targeting Nrf2 locus for knock-out via Abbie1 Editing. Abbie1 transfection targeting exon 2 of Nrf2 using guide NrF2-sgRNA 2 and 3 showed integration of donor at targeted region. Unique bands are identified as 1-8.

[0117] FIG. 6 shows theoretical data generated by Abbie1 gene editing. Representation of DNA gel electrophoresis visualizing inserted donor DNA via the Abbie1 system to target genomic material using sgRNA 1-3. Black bands represent background product due to PCR

methodology. Red bands represent unique products generated by amplifying insert and genetic material flanking the region of insert. Multiple bands represent possible multiple insertion in targeted region.

[0118] FIG. 7 shows Abbie1 Gene Editing Targeting Exon 2 of Nrf2 Using Guide Nrf2-sgRNA 3. PCR screen against exon 2 targeting Nrf2 locus for knock-out via Abbie1 Editing. Targeting exon 2 of Nrf2 using guide NrF2-sgRNA 3 suggested donor insertions as indicated by PCR primers designed to donor sequence and adjacent site to expected insertion. Unique bands are identified as 1-4

[0119] FIG. 8 shows Abbie1 Knock out of Nrf2 in pooled Hek293T Cells. (A)Western blot analysis using polyclonal antibody against 55kD isoform (Santa Cruz Bio) showing knock out of Nrf2 in pooled HEK293T poplulations. (B) GAPDH (Santa Cruz Bio) loading control.

[0120] FIG. 9 shows Abbie1 Knock out of Nrf2 in pooled Hek293T Cells. (A) Western blot analysis utilizing monoclonal antibody against Nrf2 (Abcam) showing knockout of Nrf2 pooled poplulations in HEK 293t cells. (B) GAPDH loading control. (C) Average of densitometric analysis showing decrease in expression ratios as compared to control.

[0121] Abbie1 treated cells generate a unique PCR band indicating integration of donor DNA. Phenotypic confirmation of knock out in a HEK293T pooled cell line was confirmed via western blot analysis probing for two isoforms with unique and different antibodies. ~80% knock out by integration was observed in pooled populations in under two weeks.

EXAMPLE 13: CXCR4 EDITING VIA ABBIE1

[0122] FIG. 10 shows Abbie1 Gene Editing Targeting CXCR4 Exon 2. PCR screen targeting exon 2 of CXCR4 edited via Abbie1. Four sets of primers were designed against the region of interest. Set number 2 and 4 appears to have generated unique bands suggesting integration of donor DNA at the region of interest.

EXAMPLE 14: TRANSFECTION FOR THE KNOCK-IN EXPERIMENT AT THE NRF2 LOCUS USING ABBIE1.

[0123] Note: 500 ng protein and 120ng sgRNA are used for a single reaction. The amount of DNA depends on the size of the donor constructs. Donor DNA (DNA with LTR sequences) may be incubated with ABBIE1 before, during, or after providing/transfecting/electroporating to the cells. All reactions are prepared in sterile biosafety cabinet.

[0124] Day 1: Human embryonic kidney (HEK 293T) Cells were seeded into 24-well culture plate (Corning) at 200,000 HEK293T cells (ATCC) per well in 500 µL DMEM (Gibco)

supplemented with 10% fetal bovine serum (Omega Scientific). Cells were allowed to recover for 24 hours.

[0125] Day 2: ABBIE1 Preparation:

Tube 1:
Purified ABBIE1 protein (SEQ ID NO: 58) and donor DNA (SEQ ID NO: 101) in a reduced-serum transfection medium (OptiMEM, Life Technologies) at 1:1 molar ratio for 10 minutes at room temperature. Add the sgRNA to the 1.3-fold molar excess (approximately 120 ng) to the protein/DNA complex and continue the incubation for additional 10 minutes at room temperature. The volume of this mixture is 25 µL.

Tube 2:
2µL of transfection reagent (RNAiMAX, Life Technologies) was added to 23 µL of OptiMEM. And allowed to incubate for 10 minutes at room temperature.

[0126] Combined Tube 1 and Tube 2 (50µl final volume) and incubated for 15 minutes at room temperature.

[0127] Added the entire 50µL transfection mixture to the well.

[0128] Half of the pooled edited cells were harvested 48 hours after transfection for the verification of the genomic DNA editing in a pooled population. Verification of edited genome was performed by polymerase chain reaction (PCR). We performed PCR against the targeted region as described below (See PCR protocol) the remainder was seeded onto 6 cm culture dishes (Corning) and allowed to recover for 48 hours.

[0129] Day 5: Screening of phenotypic changes via western blotting.

[0130] Standard western blot analysis was performed for NrF2 isoforms using primary antibodies targeting 55kD isoform (Santa Cruz Biotechnology, sc-722) as well as 98kD isoform (Abcam, ab-62352). GAPDH (Santa Cruz Biotechnology, sc-51907)

EXAMPLE 15: PCR CONDITIONS FOR DETECTION OF GENE EDITING USING ABBIE1 FOR NRF2 AND CXCR4 LOCUS.

[0131] Accession number for human Nrf2

Uniprot: Q16236

Ensembl gene ID: ENSG00000116044

Editing target sequences and PAMs for Nrf2 (exon 2): Used for sgRNA design 1-3.

GCGACGGAAAGAGTATGAGC TGG

TATTTGACTTCAGTCAGCGA CGG

TGGAGGCAAGATATAGATCT TGG

Primer Key for Detection of Integration at Nrf2 Target

Primer Set 1: Primer 1:5'-GTGTTAATTTCAAACATCAGCAGC-3', Primer 2: 5'-GACAAGACATCCTTGATTTG-3'

Primer Set 2: Primer 1:5'-GAGGTTGACTGTGTAAATG-3', Primer 2: 5'-GATACCAGAGTCACACAACAG-3'

Primer Set 3: Primer 1: 5'-TCTACATTAATTCTCTTGTGC-3', Primer 2:5'-GATACCAGAGTCACACAACAG-3'

Accession number for human CXCR4

Uniprot P61073

Ensembl gene ID: ENSG00000121966

Editing target sequence and PAM for CXCR4 (Exon 2): Used for sgRNA design1.

GGGCAATGGATTGGTCATCC TGG

Primer Key for Detection of Integration at CXCR4 Target

Primer Set 1: Primer 1: 5'- TCTACATTAATTCTCTTGTGC-3', Primer 2: 5'-GACAAGACATCCTTGATTTG-3'

Primer Set 2: Primer 1: 5'- TCTACATTAATTCTCTTGTGC-3', Primer 2: 5'-GATACCAGAGTCACACAACAG -3'

Primer Set 3: Primer 1: 5'- GAGGTTGACTGTGTAAATG -3', Primer 2: 5'-GACAAGACATCCTTGATTTG-3'

Primer Set 4: Primer 1: 5'- GAGGTTGACTGTGTAAATG -3', Primer 2: 5'-GATACCAGAGTCACACAACAG -3'

PCR Cycling conditions used for detection of integrated donor DNA

*Note annealing temperatures will vary depending on primer sequence

1. Initial denaturation:	4 min	94°C
2. denaturation:	30 sec	94°C
3. annealing:	30 sec	55°C
4. extension:	30 sec	72°C

5. go to step 2:	40 cycles	
6. final extension:	4 min	72°C
7. final hold:	∞	4°C

Avi-tagged Cas9 for biotinylation

Sequence of the avi-tag used for Cas9 biotinylation

Amino acid seqeunce:
G G D L E G S G L N D I F E A Q K I E W H E *

Nucleic acid sequence:
GGCGGCGACCTCGAGGGTAGCGGTCTGAACGATATTTTTGAAGCGCA
GAAAATTGAATGGCATGAATAA

First Underlined section = Cas9 C-terminus

Italicized section = restriction site/linker

Second underlined section = avi-tag (biotinylation site highlighted)

EXAMPLE 16: EXPRESSION PROTOCOL FOR ABBIE1 FUSION PROTEIN.

[0132] Transformation of expression construct containing full-length fusion protein (SEQ ID NO: 57).

[0133] Take competent E. coli cells from -80°C freezer.

[0134] Turn on water bath to 42°C.

[0135] Put competent cells in a 1.5 ml tube (Eppendorf or similar). For transforming a DNA construct, use 50 ul of competent cells.

[0136] Keep tubes on ice.

[0137] Add 50 ng of circular DNA into E.coli cells. Incubate on ice for 10 min. to thaw competent cells.

[0138] Put tube(s) with DNA and E.coli into water bath at 42°C for 45 seconds. Put tubes back on ice for 2 minutes to reduce damage to the E.coli cells.

[0139] Add 1 ml of LB (with no antibiotic added). Incubate tubes for 1 hour at 37 oC. (Can incubate tubes for 30 minutes

Spread about 100 ul of the resulting culture on LB plates with appropriate antibiotic

Pick colonies about 12-16 hours later.

INNOCULATION AND EXPANSION

[0140]

Innoculate a 1 liter flask containing LB and antibiotic

Allow bacterial culture to grow until 0.6 OD is achieved then induce with Isopropyl β-D-1-thiogalactopyranoside (IPTG) at a 1mM final concentration

Allow the culture to expand for 6-8 hours and centrifuge the suspended bacterial culture at a minimum of two thousand G force for 10 minutes.

[0141] Freeze pellet at -80 C for further processing at a later time

PROTEIN PREPARATION AND PURIFICATION

[0142] All steps are performed at room temperature.

[0143] Lyse the cells by 2 cycles of freeze-thaw in 20 mM Tris pH8.0, 300 mM NaCl, 0.1 mg/ml chicken egg white lysozyme. Centrifuge at 6,000 g for 15 minutes and retain the supernatant.

[0144] Load the supernatant onto a Ni-IDA agarose column equilibriated in 20 mM Tris pH8.0, 300 mM sodium chloride. Elute the protein with a 0-to-200 mM gradient of imidazole. Identify the fractions containing the fusion protein by a 7% SDS-PAGE.

[0145] Pool the fractions and dilute with 20 mM Tris pH8.0 so that the final NaCl concentration is 50 mM. Load onto a Q-sepharose column and elute with a 0-to-500 mM gradient of sodium chloride. Identify the fractions containing the fusion protein by a 7% SDS-PAGE.

[0146] Pool the fractions and dilute with 20 mM Tris pH8.0 so that the final NaCl concentration is 100 mM. Load onto an SP-sepharose column and elute with a 0-to-500 mM gradient of sodium chloride. Identify the fractions containing the fusion protein by a 7% SDS-PAGE.

[0147] Pool the fractions, measure the concentration by its UV absorbance at 280 nm, and concentrate by a centrifugal filter to the final concentration of 400 µg/ml. Add glycerol to the

final concentration of 50%. Store at -20°C.

[0148] While certain embodiments 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 disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

SEQUENCES OF THE DISCLOSURE

[0149] For each sequence provided below, the following information is provided: type of sequence (nucleic acid or amino acid), source (e.g. E. coli), length, and identification number (if available).

[0150] A first polynucleotide of the disclosure can encode, for example, a Cas9, Cpfl, TALE, or ZnFn protein. A second polynucleotide of the disclosure can encode, for example, an integrase, transposase, or recombinase. Listed below are exemplary first and second polynucleotide sequences and protein sequences, along with exemplary linker sequences, that can be used in the compositions (constructs, fusion proteins) and methods described herein. Other polynucleotide sequences, protein sequences, or linker sequences may be provided in the disclosure that are not listed in Table 1 below, but can be used in the compositions (constructs, fusion proteins) and methods described herein. For example, SEQ ID NO: 49, SEQ ID NO: 57, SEQ ID NO: 58, and/or portions thereof.

[0151] A linker can be any length, for example, 3 to 300 nucleotides in length, 6 to 60 nucleotides in length, or any length that will allow the first and second polynucleotide to be fused. A polypeptide can be made by an organism, e.g. E. coli or be made synthetically, or a combination of both.

[0152] Exemplary nucleic acid sequences: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 27-47, 49, 55, 56, 57, 62, 64, 66, 68, 70, 79, 82, and 83.

[0153] Exemplary amino acid sequences: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 25, 26, 48, 50, 52, 58, 63, 65, 67, 69, 71, 72-78, and 80.

TABLE 1: FIRST PROTEIN, SECOND PROTEIN, OR LINKER

first polynucleotide or protein sequence SEQ ID NO:	second polynucleotide or protein sequence SEQ ID NO:	linker sequence SEQ ID NO: or sequence
1-14, 27-46, 50, 52, 56, 57, 68, 69, 72-78, 86-92, 200-253	15-26, 47, 48, 55, 57, 62-67, 70, 71, 79, 80	51, 54, 61, GGS

TABLE 2: PARTIAL LIST OF SEQUENCES

Gene (protein)	Bacteria/Virus	DNA sequence	Protein sequence	SEQ ID (DNA, protein)
Cas9	S. thermophilus	HQ712120.1	Q03JI6.1	SEQ ID NOS: 1, 2
	P. multocida		Q9CLT2.1	SEQ ID NOS: 3, 4
	S. mutans		Q8DTE3.1	SEQ ID NOS: 5, 6
	N. meningitides		C9X1G5.1	SEQ ID NOS: 7, 8
	S. mitis		KJQ69483.1	SEQ ID NOS: 9, 10
	S. macacae		EHJ52063.1	SEQ ID NOS: 11,12
	Staphylococcus Aureus		KKJ92487.1	SEQ ID NOS: 49, 50
	S. pyogenes		AFV37892.1	SEQ ID NOS: 13, 14
Integrase	HIV1		ABR68182.1	SEQ ID NOS: 15, 16
	Simian T-lymphocyte virus		AAA47841.1	SEQ ID NOS: 17, 18
	S. pneumonia		CBW38769.1	SEQ ID NOS: 19, 20
	E. coli		CAA41325.1	SEQ ID NOS: 21, 22
	Lentivirus			SEQ ID NOS: 47, 48
Recombinase	Thermoanaerobacterium phage		YP_006546326.1	SEQ ID NOS: 23

Gene (protein)	Bacteria/Virus	DNA sequence	Protein sequence	SEQ ID (DNA, protein)
				24

ADDITIONAL SEQUENCES

[0154]

SEQ ID NO: 1

NAME: S.thermophilus Csn1 cds HQ712120.1

SEQUENCE:

ATGACTAAGCCATACTCAATTGGACTTGATATTGGAACGAATAGTGTTGGAT
GGGCTGTAATAACTGATAATTACAAGGTTCCGTCTAAAAAAATGAAAGTCTT
AGGAAATACGAGTAAAAAGTATATCAAAAAGAACCTGTTAGGTGTATTACTC
TTTGACTCTGGAATCACAGCAGAAGGAAGAAGATTGAAGCGTACTGCAAGAA
GACGTTATACTAGACGCCGTAATCGTATCCTTTATTTGCAGGAAATTTTTAGC
ACGGAGATGGCTACATTAGATGATGCTTTCTTTCAAAGACTTGACGATTCGTT
TTTAGTTCCTGATGATAAACGTGATAGTAAGTATCCGATATTTGGAAACTTAG
TAGAAGAAAAAGTCTATCATGATGAATTTCCAACTATCTATCATTTAAGGAA
ATATTTAGCAGATAGTACTAAAAAAGCAGATTTGCGTCTAGTTTATCTTGCAT
TGGCTCATATGATTAAATATAGAGGTCACTTCTTAATTGAAGGAGAGTTTAAT
TCAAAAAATAATGATATTCAGAAGAATTTTCAAGACTTTTTGGACACTTATAA
TGCTATTTTTGAATCGGATTTATCACTTGAGAATAGTAAACAACTTGAGGAAA
TTGTTAAAGATAAGATTAGTAAATTAGAAAAGAAAGATCGTATTTTAAAACT
CTTCCCTGGGGAGAAGAATTCGGGGATTTTTTCAGAGTTTCTAAAGTTGATTG
TAGGAAATCAAGCTGATTTTAGGAAATGTTTTAATTTAGACGAAAAAGCCTC
CTTACATTTTTCCAAAGAAAGCTATGATGAAGATTTAGAGACTTTGTTAGGTT
ATATTGGAGATGATTACAGTGATGTCTTTCTCAAAGCAAAGAAACTTTATGAT
GCTATTCTTTTATCGGGTTTTCTGACTGTAACTGATAATGAGACAGAAGCACC
TCTCTCTTCTGCTATGATAAAGCGATATAATGAACACAAAGAAGATTTAGCGT
TACTAAAGGAATATATAAGAAATATTTCACTAAAAACGTATAATGAAGTATT
TAAAGATGACACCAAAAATGGTTATGCTGGTTATATTGATGGAAAAACAAAT
CAGGAAGATTTCTACGTATATCTAAAAAACCTATTGGCTGAATTTGAAGGTG
CGGATTATTTTCTTGAAAAAATTGATCGAGAAGATTTTTTGAGAAAGCAACGT
ACATTTGACAATGGTTCGATACCATATCAGATTCATCTTCAAGAAATGAGAG

CAATTCTTGATAAGCAAGCTAAATTTTATCCTTTCTTGGCTAAAAATAAAGAA

AGAATCGAGAAGATTTTAACCTTCCGAATTCCTTATTATGTAGGTCCACTTGC
GAGAGGGAATAGTGATTTTGCCTGGTCAATAAGAAAACGAAATGAAAAAATT
ACACCTTGGAATTTTGAGGACGTTATTGACAAAGAATCTTCGGCAGAGGCTTT
CATTAATCGAATGACTAGTTTTGATTTGTATTTGCCAGAAGAGAAGGTACTTC
CAAAGCATAGTCTCTTATACGAAACTTTTAATGTATATAATGAATTAACAAAA
GTTAGATTTATTGCCGAAAGTATGAGAGATTATCAATTTTTAGATAGTAAGCA
GAAGAAAGATATTGTTAGACTTTATTTTAAAGATAAAAGGAAAGTTACTGAT
AAGGATATTATTGAATATTTACATGCAATTTATGGGTATGATGGAATTGAATT
AAAAGGCATAGAGAAACAGTTTAATTCTAGTTTATCTACTTATCACGATCTTT
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SEQ ID NO: 2

SEQUENCE:

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SEQ ID NO: 3

NAME: P.multocida Cas9

SEQUENCE:
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SEQ ID NO: 4

SEQUENCE:

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SEQ ID NO: 5

NAME: S.mutans Cas9

SEQUENCE:
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SEQ ID NO: 6

SEQUENCE:

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SEQ ID NO: 7

NAME: N.meningitides Cas9

SEQUENCE:
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SEQ ID NO: 8

SEQUENCE:

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WQLKAAALDKKLTPLEWSAVLLHLIKHKGYLSQKKNEGETADKELGALLKGVA
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SEQ ID NO: 9
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TCTTTACGAGACTCGGATTGACTTAAGCAAACTGGGAGAAGACTGA

SEQ ID NO: 10

NAME: gi|777888062|gb|KJQ69483.1| CRISPR-associated endonuclease Cas9

[Streptococcus mitis]

SEQUENCE:
MNNNNYSIGLDIGTNSVGWAVITDDYKVPSKKMKVLGNTDKHFIKKNLIGALLF
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KLGED

SEQ ID NO: 11

SEQUENCE:

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SEQ ID NO: 12

NAME: gi|357584860|gb|EHJ52063.1| CRISPR-associated protein Cas9/Csn1, subtype II/NMEMI [Streptococcus macacae NCTC 11558]

SEQUENCE:
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SEQ ID NO: 13
SEQUENCE:
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TTAAGCAATTAAAAGAAGATTATTTCAAAAAAATAGAATGTTTTGATAGTGT
TGAAATTTCAGGAGTTGAAGATAGATTTAATGCTTCATTAGGTACCTACCATG
ATTTGCTAAAAATTATTAAAGATAAAGATTTTTTGGATAATGAAGAAAATGA
AGATATCTTAGAGGATATTGTTTTAACATTGACCTTATTTGAAGATAGGGAGA
TGATTGAGGAAAGACTTAAAACATATGCTCACCTCTTTGATGATAAGGTGAT
GAAACAGCTTAAACGTCGCCGTTATACTGGTTGGGGACGTTTGTCTCGAAAA
TTGATTAATGGTATTAGGGATAAGCAATCTGGCAAAACAATATTAGATTTTTT
GAAATCAGATGGTTTTGCCAATCGCAATTTTATGCAGCTGATCCATGATGATA
GTTTGACATTTAAAGAAGACATTCAAAAAGCACAAGTGTCTGGACAAGGCGA
TAGTTTACATGAACATATTGCAAATTTAGCTGGTAGCCCTGCTATTAAAAAAG
GTATTTTACAGACTGTAAAAGTTGTTGATGAATTGGTCAAAGTAATGGGGCG
GCATAAGCCAGAAAATATCGTTATTGAAATGGCACGTGAAAATCAGACAACT
CAAAAGGGCCAGAAAAATTCGCGAGAGCGTATGAAACGAATCGAAGAAGGT
ATCAAAGAATTAGGAAGTCAGATTCTTAAAGAGCATCCTGTTGAAAATACTC
AATTGCAAAATGAAAAGCTCTATCTCTATTATCTCCAAAATGGAAGAGACAT
GTATGTGGACCAAGAATTAGATATTAATCGTTTAAGTGATTATGATGTCGATC
ACATTGTTCCACAAAGTTTCCTTAAAGACGATTCAATAGACAATAAGGTCTTA
ACGCGTTCTGATAAAAATCGTGGTAAATCGGATAACGTTCCAAGTGAAGAAG
TAGTCAAAAAGATGAAAAACTATTGGAGACAACTTCTAAACGCCAAGTTAAT
CACTCAACGTAAGTTTGATAATTTAACGAAAGCTGAACGTGGAGGTTTGAGT
GAACTTGATAAAGCTGGTTTTATCAAACGCCAATTGGTTGAAACTCGCCAAA
TCACTAAGCATGTGGCACAAATTTTGGATAGTCGCATGAATACTAAATACGA
TGAAAATGATAAACTTATTCGAGAGGTTAAAGTGATTACCTTAAAATCTAAA
TTAGTTTCTGACTTCCGAAAAGATTTCCAATTCTATAAAGTACGTGAGATTAA
CAATTACCATCATGCCCATGATGCGTATCTAAATGCCGTCGTTGGAACTGCTT
TGATTAAGAAATATCCAAAACTTGAATCGGAGTTTGTCTATGGTGATTATAAA
GTTTATGATGTTCGTAAAATGATTGCTAAGTCTGAGCAAGAAATAGGCAAAG
CAACCGCAAAATATTTCTTTTACTCTAATATCATGAACTTCTTCAAAACAGAA
ATTACACTTGCAAATGGAGAGATTCGCAAACGCCCTCTAATCGAAACTAATG
GGGAAACTGGAGAAATTGTCTGGGATAAAGGGCGAGATTTTGCCACAGTGCG

CAAAGTATTGTCCATGCCCCAAGTCAATATTGTCAAGAAAACAGAAGTACAG
ACAGGCGGATTCTCCAAGGAGTCAATTTTACCAAAAAGAAATTCGGACAAGC
TTATTGCTCGTAAAAAAGACTGGGATCCAAAAAAATATGGTGGTTTTGATAG
TCCAACGGTAGCTTATTCAGTCCTAGTGGTTGCTAAGGTGGAAAAAGGGAAA
TCGAAGAAGTTAAAATCCGTTAAAGAGTTACTAGGGATCACAATTATGGAAA
GAAGTTCCTTTGAAAAAAATCCGATTGACTTTTTAGAAGCTAAAGGATATAA
GGAAGTTAAAAAAGACTTAATCATTAAACTACCTAAATATAGTCTTTTTGAGT

TAGAAAACGGTCGTAAACGGATGCTGGCTAGTGCCGGAGAATTACAAAAAG
GAAATGAGCTGGCTCTGCCAAGCAAATATGTGAATTTTTTATATTTAGCTAGT
CATTATGAAAAGTTGAAGGGTAGTCCAGAAGATAACGAACAAAAACAATTGT
TTGTGGAGCAGCATAAGCATTATTTAGATGAGATTATTGAGCAAATCAGTGA
ATTTTCTAAGCGTGTTATTTTAGCAGATGCCAATTTAGATAAAGTTCTTAGTG
CATATAACAAACATAGAGACAAACCAATACGTGAACAAGCAGAAAATATTA
TTCATTTATTTACGTTGACGAATCTTGGAGCTCCCGCTGCTTTTAAATATTTTG
ATACAACAATTGATCGTAAACGATATACGTCTACAAAAGAAGTTTTAGATGC
CACTCTTATCCATCAATCCATCACTGGTCTTTATGAAACACGCATTGATTTGA
GTCAGCTAGGAGGTGACTGA

SEQ ID NO: 14

NAME: gi|409693032|gb|AFV37892.1| CRISPR-associated protein, Csn1 family

[Streptococcus pyogenes A20]

SEQUENCE:
MDKKYSIGLDIGTNSVGWAVITDDYKVPSKKFKVLGNTDRHSIKKNLIGALLFDS
GETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEED
KKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFR
GHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRR
LENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLD
NLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDL
TLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTE
ELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKIL
TFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFD
KNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLF

KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLD
NEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRL
SRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQG
DSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQK
GQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQ
ELDINRLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMK
NYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQIL
DSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYL
NAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIM
NFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTE
VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGK
SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGR
KRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHK

HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

SEQ ID NO: 15

NAME: gi|150381361|gb|EF472760.1| HIV-1 clone 39B from USA integrase (pol) gene, partial cds

SEQUENCE:
TTTTTGGATGGAATAGATAGGGCCCAAGAAGAGCATGAGAAATATCACAATA
ATTGGAGAGCAATGGCTAGTGATTTTAACCTGCCACCTNTAGTAGCAAAGGA
GATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGA
CAAGTAGACTGTAGTCCAGGAATATGGCAACTAGATTGTACACATNTAGAAG
GAAAAGTTATCCTGGTAGCAGTNCATGTAGCCAGTGGTTATATAGAAGCAGA
AGTTATTCCAGCAGAGACAGGGCAGGAAACAGCATACTTCCTCTTAAAATTA
GCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAATGGCAGCAACTTC
ACCAGTGCTGCGNTGAAGGCCGCCTGTTGGTGGGCAGGGATCAAGCAGGAAT
TTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAGTCTATGAATAA
TGAATTAAAGAAAATTGTAGGACAAGTAAGAGATCAGGCTGAGCATCTCAAG
ACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGA
TTGGGGGGTACAGTGCAGGAGAAAGAATAGTAGACATAATAGCCACAGACA

TACAAACTAAAGAACTACAAAAAAATATTACAAAAATGCAAAATTTTCGGGT
CTATTTCAGAGACAGCAGAGATCCACTTTGGAAAGGACCAGCAAAGCTTCTC
TGGAAAGGTGAAGGGGCAGTAGTAATACAAGATACCAATGACATAAARGTA
GTGCCARGAAGAAAAGCAAAGATCATTAGAGATTATGGAAAACAGATGGCA
GGTGATGATTGTGTGGCAAGTAGACAGGNTGAGGATTAG

SEQ ID NO: 16

NAME: gi|150381362|gb|ABR68182.1| integrase, partial [Human immunodeficiency virus 1]

SEQUENCE:

FLDGIDRAQEEHEKYHNNWRAMASDFNLPPXVAKEIVASCDKCQLKGEAMHGQ
VDCSPGIWQLDCTHXEGKVILVAVHVASGYIEAEVIPAETGQETAYFLLKLAGR
WPVKTVHTDNGSNFTSAAXKAACWWAGIKQEFGIPYNPQSQGVVESMNNELKK
IVGQVRDQAEHLKTAVQMAVFIHNFKRKGGIGGYSAGERIVDIIATDIQTKELQK
NITKMQNFRVYFRDSRDPLWKGPAKLLWKGEGAVVIQDTNDIKVVPXRKAKIIR
DYGKQMAGDDCVASRQXED

SEQ ID NO: 17

NAME: gi|459980|gb|L20651.1|STLKIAPOL Simian T-cell lymphotropic virus type I integrase (pol) gene, partial cds

SEQUENCE:
GACTTGTAGAACGCTCTAATGGCATTCTTAAAACCCTATTATATAAGTACTTT
ACTGACAAACCCGACCT
ACCTATGGATAATGCTCTATCCATAGCCCTATGGACGATCAACCACCTGAATG
TGTTAACCCACTGCCAC

SEQ ID NO: 18

NAME: gi|459981|gb|AAA47841.1| integrase, partial [Simian T-lymphotropic virus 1]

SEQUENCE:
LVERSNGILKTLLYKYFTDKPDLPMDNALSIALWTINHLNVLTHCH

SEQ ID NO: 19

NAME: gi|321156784:1-1509 Streptococcus pneumoniae integrative and conjugative element ICESpn11930, strain 11930

SEQUENCE:
GAGTTTTTTTCCTTTCGTAGCAAGGGTTTAGAGCCCCTATTTTATTTTACTATT
GTCTAAACACCAAGCG
AACACCAAAACTACCATGCAATGGAAAAACCTCTGATTTGATTCTCACTTGAT
TTCACAATCTTTATATC
AAACTGTGGGTGGTATTTGACAATATCTTTTTTGATTTTTAATAGTAAATTCG
AAATAATATTTTTAGGT
GAGTAACGTGGACTAAGATGTAACAAGTCTTTGAACTCATCGACACTTAATT
CTACTTTATTGCTATTAT
CACTAGTTTCAATGAATTTTTCAATTATTCTGGAATATTTACAGGTATAACTTT
TCAATTCTTCAAAATG
GAAATTGTGATTTTCTACAAATTGATTTAAGGCTTTTACAGTATTTTCTTGTGA
ACGATTTATATTATGT
GTATAGCCCATTGTTGTCTCAAAGTTAGCGTGTCCTACTCTAGTCATAATATC
TTTCACTGCTATGTGCA
TCTCATTACTTTGAAGGTAACTAATATGCATATGCCTAAACGAATGGGGAGT
AACATGTTTTACCCACTT
AAAACCATAGTCACTTAAACAATTTGTCAATAATTTTCCTTCTATTCGTTTCA
AAATTTGACGAAAAGTG
CTTGATGTTATTGGAGAGCCGTATTCTGTTCTAAATACACTTTCAGAATGTGT
AAAAGCAGGACAGGGAT
GTTTCTCCATATAAGCATCAAACTCTTTATTTCTCTGTATTGTCCTTTTAATAG
CTTCGCTTGCAGCTTC
AGGCAAAGCTACTTCTCTAATTGAATTGAGTGTTTTAGTTGTATCAAAATGAA
ATTGTTTAACTTTTAAA
GAATGATATTCAAGTCGTTTATGAATATCCAAGATTCGTTTTTCAAAATGAAT

CAATGATATTGAAGTGCTTTATCAATATGCAAGATTCCTTTTTCAAAATCAAT
ATCTGATGGTAAAAATG
CTGCTTCACTAATTCGAATACCTGTAAGCAACAATACTATAGCAAGATCATA
ATAGTTTGCATTTCTGCA
TTGGCGTAACACATCAAAAAATGCATGTAATTCATGGATTTCTAGAAATTTAG
AATCATGTCTTTCTTTT

GCTTTACGCCTTTTCTCTAGTGAAATATCTAGTTTTACCGCAGTCATTGGAGA
AAACTTAATGACATTAT
ATAACACACCATGATTAAAAATCTTATTACAAGTACTTTTTATATGAGTCATT
GTTGAAGGCGATGCATC
ATACATTTCTAAATATTTATTGAGACTATTTTTCATCAGAAGTGGAGTAATCC
TGTCTAACAAAAAATCA
TCTCCTATAATTTTCCCAAGACGCTTCATAACCAGTAGTTCTCTCTGAATTGTT
TGTGGTTTAACAGAGA
CACACCAAGTCTGAAACCAATTTTCTTTTAACTCTCCAAATGTTGTAATCAGT
TCAGGACTATACTGACT
TTCAAATGAAGTAGTTAGTCTATCTATTTTATCAAGAACCTCTCTTTCAGCTTG
TTTCCTCGCCCTACTA
GTATTCTTAGTATAACTTACAGTTACTGATTTCCACTTT

SEQ ID NO: 20

NAME: gi|321156785|emb|CBW38769.1| Integrase [Streptococcus pneumoniae]

SEQUENCE:
MYYVTKTNSKGQPLYQVVEKYKDPLTGKWKSVTVSYTKNTSRARKQAEREVL
DKIDRLTTSFESQYSPEL
ITTFGELKENWFQTWCVSVKPQTIQRELLVMKRLGKIIGDDFLLDRITPLLMKNSL
NKYLEMYDASPSTM
THIKSTCNKIFNHGVLYNVIKFSPMTAVKLDISLEKRRKAKERHDSKFLEIHELHA
FFDVLRQCRNANYY
DLAIVLLLTGIRISEAAFLPSDIDFEKGILHIDKALQYHCLKVKQFHFDTTKTLNSIR
EVALPEAASEAI
KRTIQRNKEFDAYMEKHPCPAFTHSESVFRTEYGSPITSSTFRQILKRIEGKLLTNC
LSDYGFKWVKHVT
PHSFRHMHISYLQSNEMHIAVKDIMTRVGHANFETTMGYTHNINRSQENTVKAL
NQFVENHNFHFEELKS
YTCKYSRIIEKFIETSDNSNKVELSVDEFKDLLHLSPRYSPKNIISNLLLKIKKDIVK
YHPQFDIKIVKS
SENQIRGFSIAW

SEQ ID NO: 21

NAME: gi|43090:1-436 E.coli (Tn5086) dhfrVII gene for dihydrofolate reductase type VII and sull gene, 5' end (integrase)

SEQUENCE:
GCATGCCCGTTCCATACAGAAGCTGGGCGAACAAACGATGCTCGCCTTCCAG
AAAACCGAGGATGCGAAC
CACTTCATCCGGGGTCAGCACCACCGGCAAGCGCCGCGACGGCCGAGGTCTT
CCGATCTCCTGAAGCCAG
GGCAGATCCGTGCACAGCACCTTGCCGTAGAAGAACAGCAAGGCCGCCAAT
GCCTGACGATGCGTGGAGA
CCGAAACCTTGCGCTCGTTCGCCAGCCAGGACAGAAATGCCTCGACTTCGCT
GCTGCCCAAGGTTGCCGG
GTGACGCACACCGTGGAAACGGATGAAGGCACGAACCCAGTGGACATAAGC
CTGTTCGGTTCGTAAGCTG
TAATGCAAGTAGCGTATGCGCTCACGCAACTGGTCCAGAACCTTGACCGAAC
GCAGCGGTGGTAACGGCG
CAGTGGCGGTTTTCAT

SEQ ID NO: 22

NAME: gi|43091|emb|CAA41325.1| integrase, partial (plasmid) [Escherichia coli]

SEQUENCE:
MKTATAPLPPLRSVKVLDQLRERIRYLHYSLRTEQAYVHWVRAFIRFHGVRHPA
TLGSSEVEAFLSWLAN
ERKVSVSTHRQALAALLFFYGKVLCTDLPWLQEIGRPRPSRRLPVVLTPDEVVRI
LGFLEGEHRLFAQLL
YGTGM

SEQ ID NO: 23

>gi|397912605:40372-41898 Thermoanaerobacterium phage THSA-485A, complete genome - recombinase
ATGAATCGTGTATGTATTTATCTTAGGAAGTCCCGAGCAGACGAAGAAATAG
AAAAAGAGCTTGGACAAG

GAGAAACACTCGCAAAACATCGTAAGGCCCTTCTTAAATTTGCAAAAGAGAA
AAATTTGAACATAGTAAA
AATCAGAGAGGAAATAGTATCAGGCGAAAGCCTTATCCATAGACCTGAAATG
TTGGAATTACTAAAAGAA
GTCGAACAAGGCATGTACGATGCTGTATTATGTATGGATCTACAGCGTTTAG
GGCGTGGCAACATGCAGG
AACAAGGTCTCATTTTAGAAGCCTTTAAAAAGTCAAACACTAAAATTATAAC
GCTTCAAAAAACTTATGA
TTTGAACAATGATTTTGACGAAGAATATAGCGAATTTGAAGCATTTATGAGC

CGAAAGGAACTTAAAATG
ATAAATAGAAGGCTACAAGGTGGCAGAGTACGCTCTATTCAGGAAGGTAATT
ATTTATCACCATTGCCAC
CTTATGGTTACTTAATACACGAAGAAAAATTTTCGCGCACTCTTGTGCCTAAT
CCTGAGCAAGCTGATGT
AGTTAAAATGATTTTTGATATGTATGTCAATAAACAGATGGGGTCTAGTGCTA
TAGCGAACGAACTAAAC
AAAATGGGTTATAAGACGTATACTGGCAGGAATTGGGCTTCAAGCTCTGTAA
TAAACATACTCAAGAATC
CAGTTTACATCGGTAAAATAACGTGGAAGAAGAAGGATATAAAGAAGTCTGC
TGACCCAAATAAAAGCAA
AGATACACGTCAAAGACCACGCTCTGAATGGATTGTATCAGATGGCAAACAT
GAACCAATAGTGGGCAAA
GAGCTCTTTGCCAAGGCTCAAGAAATCATTAAAAACAAGTATCACATACCGT
ATCAGATCGTTAATGGTC
CACGTAACCCATTGGCAGGGCTTATTATATGCAAAATATGTGGCTCTAAAAT
GGTGTATAGACCCTACAA
AGATAAAGAAGCGCATATAATATGTCCAAACAAGTGCGGCAATAAAAGCAG
CAAATTTATCTATGTAGAA
AAAAGATTATTACAGGCTTTGGAGGAATGGATGCAAGGCTACGAGCTGGATC
TGCAAATAGAAGAAGATG

ACAGCTCTTTTGCAGAAGCACAAGAGAAACAAAAAGAAGCTCTTGAAAGAG
AATTGCACGAGCTGCAAAA
GCAAAAGAACAATTTACACGATTTGCTCGAGCGTGGCATATACGATATAGAT
ACATTTGTGGAAAGATCT
ACAATTGTAGCACAGAGAATAGAAGAAACACAGAAAAGTATAGATGTGCTT
GTGCAAAAAATAGAAGAAG
AAAAGAATAAAAGAGACAAAGAAAAAATACTTCCGGAAATTCGGCATGTGT
TGGATCTATATTGGAAAAC
AGACGACATTGCACAAAAAAATATGTTGTTAAAGAGCGTACTTGAAAAAGCA
GAATATCTAAAAGAAAAG
AAGCAGAGAGAAGACAACTTCGAACTTTGGATTTATCCAAAGCTGCCTGAAA
AATAG

SEQ ID NO: 24

>gi|397912662|ref|YP_006546326.1| Recombinase [Thermoanaerobacterium phage THSA-485A]
MNRVCIYLRKSRADEEIEKELGQGETLAKHRKALLKFAKEKNLNIVKIREEIVSG
ESLIHRPEMLELLKE
VEQGMYDAVLCMDLQRLGRGNMQEQGLILEAFKKSNTKIITLQKTYDLNNDFD

EEYSEFEAFMSRKELKM

INRRLQGGRVRSIQEGNYLSPLPPYGYLIHEEKFSRTLVPNPEQADVVKMIFDMY

VNKQMGSSAIANELN

KMGYKTYTGRNWASSSVINILKNPVYIGKITWKKKDIKKSADPNKSKDTRQRPR

SEWIVSDGKHEPIVGK

ELFAKAQEIIKNKYHIPYQIVNGPRNPLAGLIICKICGSKMVYRPYKDKEAHIICPN

KCGNKSSKFIYVE

KRLLQALEEWMQGYELDLQIEEDDSSFAEAQEKQKEALERELHELQKQKNNLH

DLLERGIYDIDTFVERS

TIVAQRIEETQKSIDVLVQKIEEEKNKRDKEKILPEIRHVLDLYWKTDDIAQKNML

LKSVLEKAEYLKEK

KQREDNFELWIYPKLPEK

SEQ ID NO: 25

Gin recombinase

>gi|657193240|sp|Q38199.2|GIN_BPD10 RecName: Full=Serine recombinase gin; AltName: Full=G-segment invertase; Short=Gin
MLIGYVRVSTNDQNTDLQRNALVCAGCEQIFEDKLSGTRTDRPGLKRALKRLQK

GDTLVVWKLDRLGRSM

KHLISLVGELRERGINFRSLTDSIDTSSPMGRFFFHVMGALAEMERELIIERTMAG

LAAARNKGRIGGRP

PKLTKAEWEQAGRLLAQGIPRKQVALIYDVALSTLYKKHPAKRTHIENDDRINQI

DR

SEQ ID NO: 26

Cre recombinase

>gi|375331813|dbj|BAL61207.1| Cre recombinase [Cre-expression vector pHVX2-cre]
MVQTSLLTVHQNLPALPVDATSDEVRKNLMDMFRDRQAFSEHTWKMLLSVCRS

WAAWCKLNNRKWFPAEP

EDVRDYLLYLQARGLAVKTIQQHLGQLNMLHRRSGLPRPSDSNAVSLVMRRIRK

ENVDAGERAKQALAFE

RTDFDQVRSLMENSDRCQDIRNLAFLGIAYNTLLRIAEIARIRVKDISRTDGGRML

IHIGRTKTLVSTAG

VEKALSLGVTKLVERWISVSGVADDPNNYLFCRVRKNGVAAPSATSQLSTRALE

GIFEATHRLIYGAKDD

SGQRYLAWSGHSARVGAARDMARAGVSIPEIMQAGGWTNVNIVMNYIRNLDSE

TGAMVRLLEDGD

SEQ ID NOS: 27-46

These are exemplary sequences of polynucleotides encoding the TALE repeat modules for use

in linking to integrases or recombinases as described in this invention.

SEQ ID NO: 27

NAME: NI

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGG
GAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTGTG

CCAAGCGCACGGA

SEQ ID NO: 28

NAME: NG

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGG
CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTGT
GCCAAGCGCACGGC

SEQ ID NO: 29

NAME: HD

SEQUENCE:
TTGACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAGG
AAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTGT
GCCAAGCGCACGGG

SEQ ID NO: 30

NAME: NN

SEQUENCE:
CTTACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG
AAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTGT
GCCAAGCGCACGGG

SEQ ID NO: 31

NAME: NI-NI
SEQUENCE: CTGACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGG
GAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCCTC
CAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTGC
TTCCCGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 32

NAME: NI-NG

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGG
GAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCCATTGCCTC
GAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATTG
CTGCCGGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 33

NAME: NI-HD

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGG
GAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCGATCGCAA
GCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGCT
GTTGCCTGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 34

NAME: NI-NN

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCCTCCAACATTGGCGG
GAAACAGGCACTCGAGACTGTCCAGCGCCTGCTTCCCGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCGA
GCAATAACGGCGGAAAACAGGCTTTGGAAACGGTGCAGAGGCT
CCTTCCAGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 35

NAME: NG-NI

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGG
CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCCTC
CAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTGC
TTCCCGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 36

NAME: NG-NG

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGG
CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTTTG

CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTTTG

TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCCATTGCCTC

GAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATTG

CTGCCGGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 37

NAME: NG-HD

SEQUENCE:
CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTTTG

TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCGATCGCAA

GCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGCT

GTTGCCTGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 38

NAME: NG-NN

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCCATTGCCTCGAATGGAGGGGG

CAAACAGGCGTTGGAAACCGTACAACGATTGCTGCCGGTGCTTTG

TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCGA

GCAATAACGGCGGAAAACAGGCTTTGGAAACGGTGCAGAGGCT

CCTTCCAGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 39

NAME: HD-NI

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAG

GAAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTTT

GTCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCCT

CCAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTG

CTTCCCGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 40

NAME: HD-NG

SEQUENCE:
GAAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTTT

GTCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCCATTGCCT

CGAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATT

GCTGCCGGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 41

NAME: HD-HD

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAG
GAAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTTT
GTCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCGATCGCA
AGCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGC
TGTTGCCTGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 42

NAME: HD-NN

SEQUENCE:
CTCACCCCAGAGCAGGTCGTGGCGATCGCAAGCCACGACGGAGG
AAAGCAAGCCTTGGAAACAGTACAGAGGCTGTTGCCTGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCGA
GCAATAACGGCGGAAAACAGGCTTTGGAAACGGTGCAGAGGCT
CCTTCCAGTGCTGTGCCAAGCGCACGGA

SEQ ID NO: 43

NAME: NN-NI

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG
AAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCCTC
CAACATTGGCGGGAAACAGGCACTCGAGACTGTCCAGCGCCTGC
TTCCCGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 44

NAME: NN-NG

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG
AAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTTTG
TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCCATTGCCTC
GAATGGAGGGGGCAAACAGGCGTTGGAAACCGTACAACGATTG
CTGCCGGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 45

NAME: NN-HD

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG

CTGACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG

AAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTTTG

TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCGATCGCAA

GCCACGACGGAGGAAAGCAAGCCTTGGAAACAGTACAGAGGCT

GTTGCCTGTGCTGTGCCAAGCGCACGGT

SEQ ID NO: 46

NAME: NN-NN

SEQUENCE:
CTGACCCCAGAGCAGGTCGTGGCAATCGCGAGCAATAACGGCGG

AAAACAGGCTTTGGAAACGGTGCAGAGGCTCCTTCCAGTGCTTTG

TCAGGCACACGGCCTCACTCCGGAACAAGTGGTCGCAATCGCGA

GCAATAACGGCGGAAAACAGGCTTTGGAAACGGTGCAGAGGCT

SEQ ID NO: 47

NAME: gi|71796612|gb|DQ084353.1| Ovine lentivirus isolate Ov10 integrase (pol) gene, partial cds

SEQUENCE:
CATAGTAAATGGCATCAAGATGCTATGTCATTGCAGTTAGATTTTGGGATACC

GAAAGGTGCGGCAGAAG

ATATAGTACAACAATGTGAAGTATGTCAGGAAAATAAAATGCCTAGCACCAT

CAGAGGAAGTAACAAAAG

AGGGATAGATCATTGGCAGGTGGATTATACTCATTATAAAGACAAAATAATA

TTGGTATGGGTAGAAACA

AATTCGGGA

SEQ ID NO: 48

NAME: gi|71796613|gb|AAZ41325.1| integrase, partial [Ovine lentivirus]

SEQUENCE:
HSKWHQDAMSLQLDFGIPKGAAEDIVQQCEVCQENKMPSTIRGSNKRGIDHWQ

VDYTHYKDKIILVWVET

NSG

SEQ ID NO: 49

>gb|AYLT01000127.1|:11804-12046 Staphylococcus aureus subsp. aureus SK1585 contig000127, whole genome shotgun sequence
TTATAGATAGGTTAGTGACAAAATACATTTTTCGTCTAGATTAACCGTGCCTC

TTAGATTATTAATATTT

TCGTTTAGATGTTTTTCAGAAACTTTAGCAACTTCATAATCGTTCATGTAAAG

TGTTTGGTTTTTTATTG

TATAATTAAGTAATTCATAATCTTTGTATACTTCTTTTACTTTATCTATATCAA

CATTTTCAAGAACAAG

TTTTTTTATGTTATTATAATTAAAGTTTTCCAT

SEQ ID NO: 50

>gi|669035130|gb|KFD30483.1| hypothetical protein D484_02234 [Staphylococcus aureus subsp. aureus SK1585] - s aureus cas9

MENFNYNNIKKLVLENVDIDKVKEVYKDYELLNYTIKNQTLYMNDYEVAKVSE

KHLNENINNLRGTVNLD

EKCILSLTYL

SEQ ID NO: 51

NAME: dna of linker2

SEQUENCE:

agcggcagcgaaaccccgggcaccagcgaaagcgcgaccccggaaagc

SEQ ID NO: 52

NAME: dCas9 protein

SEQUENCE:

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDS

GETAEATR

LKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIF

GNIVDEVA

YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVD

KLFIQLVQ

TYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSL

GLTPNFKSN

FDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNT

EITKAPLS

ASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEF

YKFIKPILEK

MDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNR

EKIEKILTFR

IPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKN

LPNEKVLPK

HSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQL

KEDYFKKI

ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDR

EMIEERLK

TYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR

NFMQLIHD

DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGR
HKPENIVIE
MARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYL
QNGRDMY
VDQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVK
KMKNYWRQ
LLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMN
TKYDENDK
LIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPK
LESEFVYG
DYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNG
ETGEIVWD

KGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPK
KYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKK
DLIIKLPKY
SLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQK
QLFVEQHK
HYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGA
PAAFKYFD TTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGD

SEQ ID NO: 53

NAME: NLS nucleotide with ATG

SEQUENCE:
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACA
AGGATGAC
GATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATTCACCGCGGG
GTACCT

SEQ ID NO: 54

NAME: GGS linker nucleotide

SEQUENCE:
GGGGGAAGT

SEQ ID NO: 55

NAME: Synthetic integrase

SEQUENCE:
ATGTTCCTGGACGGTATCGACAAAGCTCAGGACGAGCACGAAAAGTACCATT

CTAACTGGCGCGCCATGG
CCTCTGACTTCAATCTCCCGCCGGTTGTTGCCAAGGAGATCGTGGCTTCTTGC
GACAAGTGCCAATTGAA
GGGTGAGGCTATGCATGGTCAGGTCGATTGCTCTCCCGGTATCTGGCAGCTG
GACTGCACTCACCTCGAG
GGTAAGGTGATTCTCGTTGCTGTGCACGTGGCTTCCGGCTACATCGAGGCTGA
GGTCATCCCGGCTGAGA

CCGGTCAAGAGACTGCTTACTTCCTGCTCAAGCTGGCCGGCCGTTGGCCAGTT
AAGACTATTCACACTGA
TAACGGTTCTAACTTTACTTCCGCAACTGTGAAAGCTGCATGCTGGTGGGCCG
GCATTAAACAAGAGTTC
GGAATTCCGTATAACCCGCAGTCTCAGGGCGTTGTCGAGTCTATGAACAAGG
AGCTCAAAAAGATCATTG
GTCAAGTCCGTGACCAAGCTGAGCACCTTAAGACCGCTGTGCAGATGGCTGT
TTTTATTCATAACTTCAA
GCGTAAGGGTGGTATCGGTGGTTATAGCGCTGGTGAGCGTATCGTAGACATC
ATCGCTACTGATATCCAG
ACAAAGGAGCTGCAGAAGCAGATCACTAAGATCCAGAACTTCCGTGTGTACT
ATCGGGACTCTAGGAACC
CGCTCTGGAAGGGTCCTGCTAAACTGCTGTGGAAGGGAGAGGGTGCTGTTGT
TATCCAGGACAACTCTGA
TATCAAGGTGGTTCCGCGTCGTAAGGCTAAAATTATCCGCGACTACGGCAAG
CAAATGGCTGGAGACGAC
TGCGTTGCTAGCCGTCAAGACGAAGACTAA

SEQ ID NO: 56

NAME: dCas9 nucleotide with ATG

SEQUENCE:
ATGGATAAAAAGTATTCTATTGGTTTAGCTATCGGCACTAATTCCGTTGGATG
GGCTGTCA
TAACCGATGAATACAAAGTACCTTCAAAGAAATTTAAGGTGTTGGGGAACAC
AGACCGTC
ATTCGATTAAAAAGAATCTTATCGGTGCCCTCCTATTCGATAGTGGCGAAACG
GCAGAGG
CGACTCGCCTGAAACGAACCGCTCGGAGAAGGTATACACGTCGCAAGAACC
GAATATGTT
ACTTACAAGAAATTTTTAGCAATGAGATGGCCAAAGTTGACGATTCTTTCTTT
CACCGTTT
GGAAGAGTCCTTCCTTGTCGAAGAGGACAAGAAACATGAACGGCACCCCATC

TTTGGAAA
CATAGTAGATGAGGTGGCATATCATGAAAAGTACCCAACGATTTATCACCTC
AGAAAAAA
GCTAGTTGACTCAACTGATAAAGCGGACCTGAGGTTAATCTACTTGGCTCTTG
CCCATATG
ATAAAGTTCCGTGGGCACTTTCTCATTGAGGGTGATCTAAATCCGGACAACTC
GGATGTC
GACAAACTGTTCATCCAGTTAGTACAAACCTATAATCAGTTGTTTGAAGAGA
ACCCTATA
AATGCAAGTGGCGTGGATGCGAAGGCTATTCTTAGCGCCCGCCTCTCTAAAT
CCCGACGG
CTAGAAAACCTGATCGCACAATTACCCGGAGAGAAGAAAAATGGGTTGTTCG
GTAACCTT
ATAGCGCTCTCACTAGGCCTGACACCAAATTTTAAGTCGAACTTCGACTTAGC
TGAAGAT
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TCAATGAT
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GCAGGTTA
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AGCGGACTTTCGACAACGGTAGCATTCCACATCAAATCCACTTAGGCGAATT
GCATGCTA

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GATTGAGA
AAATCCTAACCTTTCGCATACCTTACTATGTGGGACCCCTGGCCCGAGGGAA
CTCTCGGTT
CGCATGGATGACAAGAAAGTCCGAAGAAACGATTACTCCATGGAATTTTGAG
GAAGTTGT
CGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGAC
AAGAATTT

ACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACA
GTGTACAA
TGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTT
CTAAGCGG
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GACAGTTA
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GATAATTA
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CGCTCACC
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GCTCGGAT
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TAACTTAA

CTAAAGCTGAGAGGGGTGGCTTGTCTGAACTTGACAAGGCCGGATTTATTAA
ACGTCAGC

TCGTGGAAACCCGCCAAATCACAAAGCATGTTGCACAGATACTAGATTCCCG
AATGAATA

CGAAATACGACGAGAACGATAAGCTGATTCGGGAAGTCAAAGTAATCACTTT
AAAGTCA

AAATTGGTGTCGGACTTCAGAAAGGATTTTCAATTCTATAAAGTTAGGGAGA
TAAATAAC

TACCACCATGCGCACGACGCTTATCTTAATGCCGTCGTAGGGACCGCACTCAT
TAAGAAA

TACCCGAAGCTAGAAAGTGAGTTTGTGTATGGTGATTACAAAGTTTATGACG
TCCGTAAG

ATGATCGCGAAAAGCGAACAGGAGATAGGCAAGGCTACAGCCAAATACTTC
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AACATTATGAATTTCTTTAAGACGGAAATCACTCTGGCAAACGGAGAGATAC
GCAAACGA

CCTTTAATTGAAACCAATGGGGAGACAGGTGAAATCGTATGGGATAAGGGCC
GGGACTTC

GCGACGGTGAGAAAAGTTTTGTCCATGCCCCAAGTCAACATAGTAAAGAAAA
CTGAGGTG

CAGACCGGAGGGTTTTCAAAGGAATCGATTCTTCCAAAAAGGAATAGTGATA
AGCTCATC

GCTCGTAAAAAGGACTGGGACCCGAAAAAGTACGGTGGCTTCGATAGCCCTA
CAGTTGCC

TATTCTGTCCTAGTAGTGGCAAAAGTTGAGAAGGGAAAATCCAAGAAACTGA
AGTCAGTC

AAAGAATTATTGGGGATAACGATTATGGAGCGCTCGTCTTTTGAAAAGAACC
CCATCGAC

TTCCTTGAGGCGAAAGGTTACAAGGAAGTAAAAAAGGATCTCATAATTAAAC
TACCAAAG

TATAGTCTGTTTGAGTTAGAAAATGGCCGAAAACGGATGTTGGCTAGCGCCG
GAGAGCTT

CAAAAGGGGAACGAACTCGCACTACCGTCTAAATACGTGAATTTCCTGTATT
TAGCGTCC

CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTT
TTGTTGAG

CAGCACAAACATTATCTCGACGAAATCATAGAGCAAATTTCGGAATTCAGTA
AGAGAGTC

ATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACA
GGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCT
CGGCGCTC
CAGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTC
TACCAAGG

AGGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAAC
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SEQ ID NO: 57

NAME: ABBIE1 (NLS-linker1-Integrase-linker2-dCas9) -DNA sequence

SEQUENCE:
ATGGACTACAAAGACCATGACGGTGATTATAAAGATCATGACATCGATTACA
AGGATGAC
GATGACAAGATGGCCCCCAAGAAGAAGAGGAAGGTGGGCATTCACCGCGGG
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GGGGGAAGTATGTTCCTGGACGGTATCGACAAAGCTCAGGACGAGCACGAA
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TGTTGCCAAGGAGATCGTGGCTTCTTGCGACAAGTGCCAATTGAA
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GGTAAGGTGATTCTCGTTGCTGTGCACGTGGCTTCCGGCTACATCGAGGCTGA
GGTCATCCCGGCTGAGA
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AAGACTATTCACACTGA
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GCGTAAGGGTGGTATCGGTGGTTATAGCGCTGGTGAGCGTATCGTAGACATC
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ACAAAGGAGCTGCAGAAGCAGATCACTAAGATCCAGAACTTCCGTGTGTACT
ATCGGGACTCTAGGAACC
CGCTCTGGAAGGGTCCTGCTAAACTGCTGTGGAAGGGAGAGGGTGCTGTTGT
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TATCAAGGTGGTTCCGCGTCGTAAGGCTAAAATTATCCGCGACTACGGCAAG

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TGCGTTGCTAGCCGTCAAGACGAAGACagcggcagcgaaaccccgggcaccagcgaaagcgcga
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TGAAGAT

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TGGCACAA

ATTGGAGATCAGTATGCGGACTTATTTTTGGCTGCCAAAAACCTTAGCGATGC
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GCAGGTTA

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GCATGCTA
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CTCTCGGTT
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CGATAAAGGTGCGTCAGCTCAATCGTTCATCGAGAGGATGACCAACTTTGAC
AAGAATTT
ACCGAACGAAAAAGTATTGCCTAAGCACAGTTTACTTTACGAGTATTTCACA
GTGTACAA
TGAACTCACGAAAGTTAAGTATGTCACTGAGGGCATGCGTAAACCCGCCTTT
CTAAGCGG
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GACAGTTA
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GGGTAGAAGATCGATTTAATGCGTCACTTGGTACGTATCATGACCTCCTAAA
GATAATTA
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AGTGTTGA
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CGCTCACC
TGTTCGACGATAAGGTTATGAAACAGTTAAAGAGGCGTCGCTATACGGGCTG
GGGACGAT
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TGACTCTTT
AACCTTCAAAGAGGATATACAAAAGGCACAGGTTTCCGGACAAGGGGACTC
ATTGCACG
AACATATTGCGAATCTTGCTGGTTCGCCAGCCATCAAAAAGGGCATACTCCA
GACAGTCA
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TGTAATCG

AGATGGCACGCGAAAATCAAACGACTCAGAAGGGGCAAAAAAACAGTCGAG

AGCGGAT

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TGGAAGGG

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GATGCCAT

TGTACCCCAATCCTTTTTGAAGGACGATTCAATCGACAATAAAGTGCTTACAC

GCTCGGAT

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ATGAAGA

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TACCAAAG

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CATTACGAGAAGTTGAAAGGTTCACCTGAAGATAACGAACAGAAGCAACTTT
TTGTTGAG
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AGAGAGTC
ATCCTAGCTGATGCCAATCTGGACAAAGTATTAAGCGCATACAACAAGCACA
GGGATAAA
CCCATACGTGAGCAGGCGGAAAATATTATCCATTTGTTTACTCTTACCAACCT
CGGCGCTC
CAGCCGCATTCAAGTATTTTGACACAACGATAGATCGCAAACGATACACTTC
TACCAAGG
AGGTGCTAGACGCGACACTGATTCACCAATCCATCACGGGATTATATGAAAC
TCGGATAG ATTTGTCACAGCTTGGGGGTGACTAA

SEQ ID NO: 58

NAME: Translation of ABBIE1 (A Binding Based Integrase Editor)

SEQUENCE:
Met D Y K D H D G D Y K D H D I D Y K D D D D K Met A P K K K R K V G I H R
G V P G G S Met F L D G I D K A Q D E H E K Y H S N W R A Met A S D F N L P P
V V A K E I V A S C D K C Q L K G E A Met H G Q V D C S P G I W Q L D C T H L
E G K V I L V A V H V A S G Y I E A E V I P A E T G Q E T A Y F L L K L A G R W
P V K T I H T D N G S N F T S A T V K A A C W W A G I K Q E F G I P Y N P Q S Q
G V V E S Met N K E L K K I I G Q V R D Q A E H L K T A V Q Met A V F I H N F
K R K G G I G G Y S A G E R I V D I I A T D I Q T K E L Q K Q I T K I Q N F R V Y
Y R D S R N P L W K G P A K L L W K G E G A V V I Q D N S D I K V V P R R K A
K I I R D Y G K Q Met A G D D C V A S R Q D E D S G S E T P G T S E S A T P E S
Met D K K Y S I G L A I G T N S V G W A V I T D E Y K V P S K K F K V L G N T
D R H S I K K N L I G A L L F D S G E T A E A T R L K R T A R R R Y T R R K N R
I C Y L Q E I F S N E Met A K V D D S F F H R L E E S F L V E E D K K H E R H P I

E G N I V D E V A Y H E K Y P T I Y H L R K K L V D S T D K A D L R L I Y L A L

FGNIVDEVAYHEKYPTIYHLKKKLVDSTDKADLRLIYLAL
AHMetIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFE
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FGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDN
LLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLS
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NFEEVVDKGASAQSFIERMetTNFDKNLPNEKVLPKHSLLY
EYFTVYNELTKVKYVTEGMetRKPAFLSGEQKKAIVDLLF
KTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGT
YHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMetIE
ERLKTYAHLFDDKVMetKQLKRRRYTGWGRLSRKLINGIR
DKQSGKTILDFLKSDGFANRNFMetQLIHDDSLTFKEDIQK
AQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVK
VMetGRHKPENIVIEMetARENQTTQKGQKNSRERMetKRIEE
GIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMetYV
DQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNR
GKSDNVPSEEVVKKMetKNYWRQLLNAKLITQRKFDNLTK
AERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMetNT
KYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNY
HHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVR
KMetIAKSEQEIGKATAKYFFYSNIMetNFFKTEITLANGEIR
KRPLIETNGETGEIVWDKGRDFATVRKVLSMetPQVNIVKK
TEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSP
TVAYSVLVVAKVEKGKSKKLKSVKELLGITIMetERSSFEK
NPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMetLA
SAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQ
KQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAY

NKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKR
YTSTKEVLDATLIHQSITGLYETRIDLSQLGGDStop

For donor DNA (att sites of LTR regions for integrase recognition).

SEQ ID NO: 59

NAME: U3att

SEQUENCE:
ACTGGAAGGGCTAATTCACTCCCAAAGAA

SEQ ID NO: 60

NAME: U5att

SEQUENCE:
GACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGT

NLS-linker1-Integrase-linker2-dCas9, or Integrase-linker1-NLS-linker2-dCas9 or Integrase-linker2-dCas9-linker1-NLS or Integrase-linker2-dCas9-NLS

Linker 1 = GGS

SEQ ID NO: 61

NAME: Linker 2

SEQUENCE:
SGSETPGTSESATPES

SEQ ID NO: 62

NAME: MMTV integrase cDNA, gb|AF071010.1|:16-1113 Mouse mammary tumor virus putative integrase, env polyprotein, and superantigen mRNA, complete cds

SEQUENCE:
ATGACAGGAAAGTGGCCTTGTATTTACTCCACTAACTGCAGAGATGTGTTGC
ATGGGACGGGGGGCACTG
CACCAGCCCTCGTGCTGAATTCGGCACGAGGAAATGCCTATGCAGATTCTTTA
ACAAGAATTCTGACCGC
TTTAGAGTCAGCTCAAGAAAGCCACGCACTGCACCATCAAAATGCCGCGGCG
CTTAGGTTTCAGTTTCAC
ATCACTCGTGAACAAGCACGAGAAATAGTAAAATTATGTCCAAATTGCCCCG
ACTGGGGACATGCACCAC

AACTAGGAGTAAACCCTAGGGGCCTTAAGCCCGGGGTTCTATGGCAAATGGA
TGTTACTCATGTCTCAGA
ATTTGGAAAATTAAAGTATGTACATGTGACAGTGGATACTTACTCTCATTTTA
CTTTCGCTACCGCCCGG
ACGGGCGAAGCAGCCAAAGATGTGTTACAACACTTGGCTCAAAGCTTTGCAT
ACATGGGCATTCCTCAAA
AAATAAAAACAGATAATGCCCCTGCCTATGTGTCTCGTTCAATACAAGAATTT
CTGGCCAGATGGAAAAT
ATCTCACGTCACGGGGATCCCTTACAATCCCCAAGGACAGGCCATTGTTGAA
CGAACGCACCAAAATATA
AAGGCACAGATTAATAAACTTCAAAAGGCTGGAAAATACTATACACCCCACC
ATCTATTGGCACATGCTC

TTTTTGTGCTGAATCATGTAAATATGGACAATCAAGGCCATACAGCGGCCGA
AAGACATTGGGGTCCAAT
CTCAGCCGATCCAAAACCTATGGTCATGTGGAAAGACCTTCTCACAGGGTCC
TGGAAAGGACCCGATGTC
CTAATAACAGCCGGACGAGGCTATGCTTGTGTTTTTCCACAGGATGCCGAATC
ACCAATCTGGGTCCCCG
ACCGGTTCATCCGACCTTTTACTGAGCGGAAAGAAGCAACGCCCACACCTGG
CACTGCGGAGAAAACGCC
GCCGCGAGATGAGAAAGATCAACAGGAAAGTCCGGAGGATGAATCTTGCCC
CCATCAAAGAGAAGACGGC
TTGGCAACATCTGCAGGCGTTAATCTCCGAAGCGGAGGAGGTTCTTAA

SEQ ID NO: 63

NAME: gi|3273866|gb|AAC24859.1| putative integrase [Mouse mammary tumor virus]

SEQUENCE:
MTGKWPCIYSTNCRDVLHGTGGTAPALVLNSARGNAYADSLTRILTALESAQES
HALHHQNAAALRFQFH
ITREQAREIVKLCPNCPDWGHAPQLGVNPRGLKPGVLWQMDVTHVSEFGKLKY
VHVTVDTYSHFTFATAR

TGEAAKDVLQHLAQSFAYMGIPQKIKTDNAPAYVSRSIQEFLARWKISHVTGIPY
NPQGQAIVERTHQNI
KAQINKLQKAGKYYTPHHLLAHALFVLNHVNMDNQGHTAAERHWGPISADPKP
MVMWKDLLTGSWKGPDV
LITAGRGYACVFPQDAESPIWVPDRFIRPFTERKEATPTPGTAEKTPPRDEKDQQE
SPEDESCPHQREDG
LATSAGVNLRSGGGS

SEQ ID NO: 64

NAME: gb|AXUN02000059.1|:5116-8850 Youngiibacter fragilis 232.1 contig_151, whole genome shotgun sequence - recombinase

SEQUENCE:
TTGAAAGATAACGATAAAAGGATGTGGGTTCAGACTTTATGGAATCCCATCA
ATGAAAGACATAAAAGTC
CACTGGATAGCCCAGAACCAGGGATTAAAGTAGCGGCCTACTGCAGAGTAAG
CATGAAAGAGGAGGAACA
ACTCCGGTCATTGGAAAACCAGGTGCATCACTATACTCATTTTATCAAAAGTA
AGCCGAATTGGAGATTT
GTAGGGGTTTATTACGATGATGGCATAAGTGCAGCCATGGCAAGTGGGAGAA
GAGGGTTCCAGCGGATTA

TCCGTCATGCTGAAGAAGGTAAGGTTGATCTGATTCTAACAAAGAATATTTC
ACGGTTTTCCAGAAATTC
CAAGGAGTTACTGGATATAATCAATCAACTGAAAGCTATCGGTGTGGGCATC
TATTTTGAGAAAGAGAAT
ATTGATACTTCAAGAGAGTACAATAAATTCCTCTTAAGCACTTATGCTGCGCT
GGCACAGGAAGAGATAG
AAACTATTTCAAACTCTACGATGTGGGGTTATGAGAAAAGGTTTCTAAAGGG
TATCCCAAAGTTCAACCG
CTTATATGGATACAAAGTCATCCATGCAGGGGATGATTCCCAATTGATTGTTC
TTGAAGATGAAGCAAAA
ATCGTAAGAATGATGTATGAACAGTACCTTCAAGGGAAGACGTTCACTGATA
TTGCAAGGGCGCTAACAG

AAGCTGGAGTGAAAACAGCCAAAGGGAAGGATGTCTGGATAGGCGGCATGA
TAAAGCATATTTTATCCAA
CGTCACCTACACCGGTAACAAGCTTACACGAGAACTGAAAAGAGATTTATTT
ACGAACAAAGTTAATAGC
GGTGAACGGGATCAGGTTTTTATAGGAAACACTCACGAACCGATCATCAGCA
ATGATATTTTCAATCTTG
TTCAAAAGAAGCTTGAGGCCAATACGAAGGAAAGAAAGCCCAGTGAGAAGC
GAGAGAAGAACCACATGTC
TGGTCGGCTACTTTGCGGAAGATGTGGATACAGTTTTACCATAATTCACAATA
GAGCTTCTCATCACTTT
AAGTGTAGCCCTAAAATCATGGGGGTCTGTGATTCTGAACTTTATCGGGATGC
GGATATTCGAGAAATGA
TGATGAGGGCAATGTATATAAAATATGACTTCACCGATGAAGACATAGTACT
AAAACTGCTGAAGGAACT
CCAGGTCATCAATCAAAATGATCACTTTGAGTTTCATAGGCTAAAGTTTATCA
CTGAAATTGAAATCGTA
AAAAGGCAGCAGGCCATTTCAGATAGATATTCAGCTATTAGCATAGAAAAAA
TGGAAGAAGAATACCGCA
CTTTTGAAAGCAAGATTGCGAAAATTGAGGATGACAGGTACATCAGAATCGA
TGCAGTGGAGTGGTTAAA
GAAAAACAAGACGCTGGATTCTTTTATCGCTCAGGTCACCACTAAAATATTG
CGAGCTTGGGTTTCCGAG
ATGACTGTTTATACACGAGATGACTTTTTAGTGCAGTGGATTGACGGAACTCA
AACTGAGATAGGAAGCT
GCGAGCATCATCTTGTGAAGGATAGAAATAGTAAGAGTTACGAGTCCGGTGA
AGAAACGAGCAGGAGGGC
CAAATTTGAAGTCAACCACATTAGTGAAACCACCGAAGGACAAGGAGAACTT

GATCTCTTAAGCAAGAGT
GCAAGTTCAAACAATGAAGATAGTAATCAACCAGAAAATAATTCTACGGGAA
AGGAGGAGCTTGAATTGA

ACTTAAACAGTAATGCAGAAATTATCAAAATTGAGCCCGGGCAAAGGGACTA
TATTATGAAGAATTTGCA
CAAGAGCCTGAGTGCAAATATGATGATGCAAAATGCTTCAGTACACACGGCA
AGTATTAACAAACCTAGA
CTTAAGACTGCTGCTTACTGCAGAATCTCAACAGATTCAGAAGAACAAAAGG
TAAGCTTGAAAACCCAAG
TAGCCTATTACACTTATCTGATTCTAAAGGATCCCCAATATGAATATGCAGGC
ATCTATGCCGATGAAGG
TATATCAGGGCGTTCTATGAAAAACCGTACAGAATTTCTCAAACTACTCGAA
GAATGTAAAGCCGGGAAT
GTGGACTTGATTTTAACCAAGTCAATCTCACGGTTTAGCAGAAACGCATTAG
ATTGCTTGGAACAGATCA
GGATGCTGAAGTCGCTGCCAAGTCCAGTTTATGTGTATTTTGAGAAAGAGAA
TATTCATACAAAAGATGA
GAAGAGTGAGCTGATGATTTCTATTTTTGGAAGTATCGCTCAGGAAGAGAGC
GTAAACATGGGAGAAGCC
ATGGCTTGGGGAAAACGGAGATATGCTGAGAGAGGGATAGTAAACCCAAGT
GTTGCACCTTATGGATATA
GAACGGTCAGAAAAGGTGAATGGGAGGTGGTTGAAGAAGAAGCTACGATCA
TTAGAAGAATTTATCGGAT
GCTCCTAAGTGGAAAGAGTATTCATGAAATCACAAAGGAGCTCTCCATGGAG
AAGATAAAGGGTCCTGGC
GGCAACGAGCAGTGGCATCTTCAAACCATTAGAAATATCTTGAGAAATGAAA
TCTATAGGGGTAACTACC
TTTATCAAAAGGCTTATATCAAGGACACGATCGAGAAGAAGGTGGTAATGAA
TCGAGGAGAACTGCCACA
GTATCTCATAGAGAATCATCATAAAGCCATTGTTGACAATGAGACCTGGGAA
AAGGTCCAGAAGGTACTA
GAAGCCAGAAGGGAAAAATATGAGAATAAAAAGTCCATAACTTATCCTGAA
GACAAAATGAAAAACGCTT

CTCTTGAAGATATTTTTACCTGTGGAGAATGTGGAAGTAAAATAGGCCATAG
AAGGAGCATCCAGAGCTC
TAATGAGATTCATTCCTGGATCTGCACAAAAGCCGCTAAGTCTTTCTTGGTGG
ACTCGTGTAAGTCCACA
AGCGTATATCAGAAGCACCTGGAGCTGCATTTTATGAAGACTCTTCTCGATAT

TAAAAAGCATCGTTCTT
TCAAAGATGAGGTGCTCACCTATATTCGAACCCAAGAAGTAGATGAAAAGGA
AGAGTGGAGAATCAAAGT
CATAGAGAAACGAATCAAAGATCTTAACAGAGAGCTTTATAATGCGGTAGAC
CAGGAGCTCAATAAAAAA
GGTCAGGACTCCAGGAAAGTTGATGAGCTCACAGAGAAAATTGTGGATCTTC
AAGAGGAATTAAAGGTGT
TTAGGGACCGAAAGGCAAAGGTTGAGGATCTTAAAGCTGAGCTTGAATGGTT
CCTAAAGAAGCTGGAAAC
CATTGATGACGCTCGAGTAAAAAGAAATGAAGGAATAGGCCACGGTGAAGA
GATCTACTTCAGAGAAGAT
ATTTTTGAAAGAATAGTAAGGAGTGCACAGCTTTATAGCGATGGAAGGATCG
TCTACGAACTAAGCCTCG
GGATCCAGTGGTTCATTGACTTTAAATACAGCGCATTTCAGAAGCTTCTTATA
AAGTGGAAGGATAAACA
AAGGGCAGAAGAAAAAGAGGCTTTTCTTGAGGGGCCGGAAGTTAAAGAGCT
GCTGGAATTTTGTAAGGAA
CCGAAGAGCTACTCTGATTTACATGCCTTCATGTGTGAGAGAAAAGAGGTGT
CTTATAGCTATTTCAGGA
AATTGGTGATAAGACCTTTGATGAAGAAAGGAAAGCTGAAGTTCACCATACC
AGAAGATGTTATGAATAG
GCATCAGAGATACACATCAATCTAA

SEQ ID NO: 65

NAME: gi|564135645|gb|ETA81829.1| recombinase [Youngiibacter fragilis 232.1]

SEQUENCE:
MKDNDKRMWVQTLWNPINERHKSPLDSPEPGIKVAAYCRVSMKEEEQLRSLEN
QVHHYTHFIKSKPNWRF
VGVYYDDGISAAMASGRRGFQRIIRHAEEGKVDLILTKNISRFSRNSKELLDIINQ
LKAIGVGIYFEKEN
IDTSREYNKFLLSTYAALAQEEIETISNSTMWGYEKRFLKGIPKFNRLYGYKVIHA
GDDSQLIVLEDEAK
IVRMMYEQYLQGKTFTDIARALTEAGVKTAKGKDVWIGGMIKHILSNVTYTGN
KLTRELKRDLFTNKVNS
GERDQVFIGNTHEPIISNDIFNLVQKKLEANTKERKPSEKREKNHMSGRLLCGRC
GYSFTIIHNRASHHF
KCSPKIMGVCDSELYRDADIREMMMRAMYIKYDFTDEDIVLKLLKELQVINQND
HFEFHRLKFITEIEIV
KRQQAISDRYSAISIEKMEEEYRTFESKIAKIEDDRYIRIDAVEWLKKNKTLDSFIA
QVTTKILRAWVSF

QVTTKILKAWVSD
MTVYTRDDFLVQWIDGTQTEIGSCEHHLVKDRNSKSYESGEETSRRAKFEVNHIS
ETTEGQGELDLLSKS
ASSNNEDSNQPENNSTGKEELELNLNSNAEIIKIEPGQRDYIMKNLHKSLSANMM
MQNASVHTASINKPR
LKTAAYCRISTDSEEQKVSLKTQVAYYTYLILKDPQYEYAGIYADEGISGRSMKN
RTEFLKLLEECKAGN
VDLILTKSISRFSRNALDCLEQIRMLKSLPSPVYVYFEKENIHTKDEKSELMISIFGS
IAQEESVNMGEA
MAWGKRRYAERGIVNPSVAPYGYRTVRKGEWEVVEEEATIIRRIYRMLLSGKSI
HEITKELSMEKIKGPG
GNEQWHLQTIRNILRNEIYRGNYLYQKAYIKDTIEKKVVMNRGELPQYLIENHH
KAIVDNETWEKVQKVL
EARREKYENKKSITYPEDKMKNASLEDIFTCGECGSKIGHRRSIQSSNEIHSWICT
KAAKSFLVDSCKST
SVYQKHLELHFMKTLLDIKKHRSFKDEVLTYIRTQEVDEKEEWRIKVIEKRIKDL
NRELYNAVDQELNKK

GQDSRKVDELTEKIVDLQEELKVFRDRKAKVEDLKAELEWFLKKLETIDDARVK
RNEGIGHGEEIYFRED
IFERIVRSAQLYSDGRIVYELSLGIQWFIDFKYSAFQKLLIKWKDKQRAEEKEAFL
EGPEVKELLEFCKE
PKSYSDLHAFMCERKEVSYSYFRKLVIRPLMKKGKLKFTIPEDVMNRHQRYTSI

SEQ ID NO: 66

NAME: gi|571264543:16423-16770 Clostridium difficile transposon Tn6218, strain Ox42 Transposase

SEQUENCE:
TTAGTCTTCAAAAGGTTTTGGACTAAATTTACTCTCGTAGTCAGGTCCAAGTG
TTTCTTCAGATTTTTTT
TTCAACCAATCCACCTGCATGGTGAGCTGGCCAACTTTTTTCGCATATTCAGC
TTTTTCCTTGCGTTCTA
AAGCGAGTTTTTCTTTCAGATTATCCTCTCGTGTGTCATTAAAAACCACGGAT
GCTTTATCGAGGAACTC
CTTCTTCCAGTTGCGGAGAAGATTCGGCTGAATATTGTTTTCGGTTGCGATTG
TATTTAAGTCTTTTTCT
CCTTTGAGCAGTTCAATCACTAATTCTGATTTGAATTTGGCAGAGAAATTTCT
TCTTGTTCGAGACAT

SEQ ID NO: 67

NAME: gi|571264559|emb|CDF47133.1| transposase [Peptoclostridium difficile]

SEQUENCE:
MSRTRRNFSAKFKSELVIELLKGEKDLNTIATENNIQPNLLRNWKKEFLDKASVV
FNDTREDNLKEKLAL
ERKEKAEYAKKVGQLTMQVDWLKKKSEETLGPDYESKFSPKPFED

SEQ ID NO: 68

NAME: gb|CP009444.1|:1317724-1320543 Francisella philomiragia strain GA01-2801, complete genome Cpf1

SEQUENCE:
ATGAATCTATATAGTAATCTAACAAATAAATATAGTTTAAGTAAAACTCTAA
GATTTGAGTTAATTCCAC

AGGGTGAAACACTTGAAAATATAAAAGCAAGAGGTTTGATTTTAGATGATGA
GAAAAGAGCTAAAGACTA
TAAAAAAGCTAAACAAATCATTGATAAATATCATCAGTTTTTTATAGAGGAG
ATATTAAGTTCGGTATGT
ATTAGCGAAGATTTATTACAAAACTATTCTGATGTTTATTTTAAACTTAAAAA
GAGTGATGATGATAATC
TACAAAAAGATTTTAAAAGTGCAAAAGATACGATAAAGAAACACATATCTAG
ATATATAAATGACTCGGA
GAAATTTAAGAATTTGTTTAATCAAAATCTTATAGATGCTAAAAAAGGGCAA
GAGTCAGATTTAATTCTA
TGGCTAAAGCAATCTAAGGATAATGGCATAGAACTATTTAAAGCTAACAGTG
ATATCACAGACATAGATG
AGGCGTTAGAAATAATCAAATCTTTTAAAGGTTGGACAACTTATTTTAAGGGT
TTTCATGAAAATAGAAA
AAATGTCTATAGTAGTGATGATATCCCTACATCTATTATTTATAGAATAGTAG
ATGATAATTTGCCTAAA
TTTATAGAAAATAAAGCTAAGTATGAGAATTTAAAAGACAAAGCTCCAGAAG
CTATAAACTATGAACAAA
TTAAAAAAGATTTGGCAGAAGAGCTAACCTTTGATATTGACTACAAAACATC
TGAAGTTAATCAAAGAGT
TTTTTCACTTGATGAAGTTTTTGAGATAGCAAACTTTAATAATTATCTAAATC
AAAGTGGTATTACTAAA
TTTAATACTATTATTGGTGGTAAATTTGTTAATGGTGAAAATACAAAGAGAA
AAGGTATAAATGAATATA
TAAATCTATACTCACAGCAAATAAATGATAAAACACTTAAAAAATATAAAAT
GAGTGTTTTATTTAAGCA
AATTTTAAGTGATACAGAATCTAAATCTTTTGTAATTGATAAGTTAGAAGATG

ATAGTGATGTAGTTACA
ACGATGCAAAGTTTTTATGAGCAAATAGCAGCTTTTAAAACATTAGAAGAAA
AGTCTATTAAGGAAACAT

TATCTTTACTATTTGATGATTTAAAAGCTCAAAAACTTGATTTGAGTAAAATT
TATTTTAAAAATGATAA
ATCTCTTACTGATCTATCACAACAAGTTTTTGATGATTATAGTGTTATTGGTAC
AGCGGTACTAGAATAT
ATAACTCAACAAGTAGCACCTAAAAATCTTGATAACCCTAGTAAGAAAGAGC
AAGATTTAATAGCCAAAA
AAACTGAAAAAGCAAAATACTTATCTCTAGAAACTATAAAGCTTGCCTTAGA
AGAATTTAATAAGTATAG
AGATATAGATAAACAGTGTAGGTTTGAAGAAATATTTGCAAGCTTTGCAGAT
ATTCCGGTGCTATTTGAT
GAAATAGCTCAAAACAAAAACAATTTGGCACAGATATCTATCAAATATCAAA
ATCAAGGTAAAAAAGACC
TGCTTCAAACTAGTGCAGAAGTAGATGTTAAAGCTATCAAGGATCTTTTGGAT
CAAACTAATAATCTCTT
GCATAAACTAAAAATATTTCATATTACGCAATCAGAAGATAAGGCAAATATT
TTAGACAAGGATGAGCAT
TTTTATTTAGTATTTGATGAGTGCTACTTTGAGCTAGCGAATATAGTGGCTCTT
TATAACAAAATTAGAA
ACTATATAACTCAAAAGCCATATAGTGATGAGAAATTTAAGCTCAATTTTGA
GAACTCAACTTTAGCCAA
TGGTTGGGATAAAAATAAAGAGCCTGACAATACGGCAATTTTATTTATCAAA
GATGATAAATATTATCTG
GGTGTGATGAACAAGAAAAATAACAAAATATTTGATGATAAAGCTATCAAAG
AAAATAAAGGTGAAGGAT
ATAAGAAAGTTGTATATAAACTTTTACCCGGTGCAAATAAAATGTTACCTAA
GGTTTTCTTTTCTGCTAA
ATCTATAAATTTTTATAATCCTAGTGAAGATATACTTAGAATAAGAAACCACT
CAACACATACAAAAAAT
GGTAGTCCTCAAAAAGGATATGAAAAACTTGAGTTTAATATTGAAGATTGCC
GAAAATTTATAGATTTTT

ATAAACATTCTATAAGTAGGCATCCAGAGTGGAAAGATTTTGGATTTAGATTT
TCTGATACTAAAAAATA
CAACTCTATAGATGAATTTTATAGAGAAGTTGAAAATCAAGGCTACAAACTA
ACTTTTGAAAATATATCA
GAAAGCTATATTGATAGTTTAGTCGATGAAGGCAAATTATACCTATTCCAAAT

CTATAATAAAGATTTCT

CAGTATATAGTAAGGGTAAACCAAATTTACATACGCTATATTGGAAGGCGTT

GTTTGATGAGAGAAATCT

CCAAGATGTAGTATATAAATTAAATGGTGAAGCAGAACTCTTCTATCGTAAA

CAATCAATACCTAAGAAA

ATCACTCACCCAGCCAAAGAGGCAATAGCTAATAAAAACAAAGATAATCCTA

AAAAAGAGAGTATTTTTG

AATATGATTTAATCAAAGATAAACGCTTTACTGAAGATAAGTTTTTCTTTCAC

TGTCCTATTACAATCAA

TTTCAAATCTAGTGGAGCTAATAAGTTTAATGATGAAATCAATTTATTGCTAA

AAGAAAAAGCAAATGAT

GTTCATATCCTAAGTATAGATAGAGGAGAAAGACATTTAGCTTACTATACTTT

GGTAGATGGTAAAGGAA

ACATTATCTGTAAGAATTAA

SEQ ID NO: 69

NAME: gi|754264888|gb|AJI57252.1| CRISPR-associated protein Cpfl, subtype PREFRAN [Francisella philomiragia]

SEQUENCE:

MKTNYHDKLAAIEKDRESARKDWKKINNIKEMKEGYLSQVVHEIAKLVIGYNAI

VVFEDLNFGFKRGRFK

VEKQVYQKLEKMLIEKLNYLVFKDNEFDKAGGVLRAYQLTAPFETFKKMGKQT

GIIYYVPADFTSKICPV

TGFVNQLYPKYESVSKSQEFFSKFDKICYNLDKGYFEFSFDYKNFGDKAAKGKW

TIASFGSRLINFRNSD

KNHNWDTREVYPTKELEKLLKDYSIEYGHGECIKAAIYAENDKKFFAKLTSILNS

ILQMRNSKTGTELDY

LISPVADVNGNFFDSRHAPKNMPQDADANGAYHIGLKGLMLLYRIKNNQDGKK

LNLVIKNEEYFEFVQNR

NKSSKI

SEQ ID NO: 70

NAME: gi|438609|gb|L21188.1|HIV1NY5A Human immunodeficiency virus type 1 integrase gene, 3' end

SEQUENCE:

TTCCTGGACGGTATCGATAAAGCTCAGGAAGAACACGAAAAATACCACTCTA

ACTGGCGCGCCATGGCTT

CTGACTTCAACCTGCCGCCGGTTGTTGCCAAGGAAATCGTGGCTTCTTGCGAC

AAATGCCAATTGAAAGG

TGAAGCTATGCATGGTCAGGTCGACTGCTCTCCAGGTATCTGGCAGCTGGACT

TGAAGCTATGCATGGTCAGGTCGACTGCTCTCCAGGTATCTGGCAGCTGGACT
GCACTCATCTCGAGGGT
AAAGTTATCCTGGTTGCTGTTCACGTGGCTTCCGGATACATCGAAGCTGAAGT
TATCCCGGCTGAAACCG
GTCAGGAAACTGCTTACTTCCTGCTTAAGCTGGCCGGCCGTTGGCCGGTTAAA
ACTGTTCACACTGACAA
CGGTTCTAACTTCACTAGTACTACTGTTAAAGCTGCATGCTGGTGGGCCGGCA
TCAAACAGGAGTTCGGG
ATCCCGTACAACCCGCAGTCTCAGGGCGTTATCGAATCTATGAACAAAGAGC
TCAAAAAAATCATTGGCC
AGGTACGTGATCAGGCTGAGCACCTGAAAACCGCGGTGCAGATGGCTGTTTT
CATCCACAACTTCAAACG
TAAAGGTGGTATCGGTGGTTACAGCGCTGGTGAACGTATCGTTGACATCATC
GCTACTGATATCCAGACT
AAAGAACTGCAGAAACAGATCACTAAAATCCAGAACTTCCGTGTATACTACC
GTGACTCTAGAGACCCGG
TTTGGAAAGGTCCTGCTAAACTCCTGTGGAAGGGTGAAGGTGCTGTTGTTATC
CAGGACAACTCTGACAT
CAAAGTGGTACCGCGTCGTAAAGCTAAAATCATTCGCGACTACGGCAAACAG
ATGGCTGGTGACGACTGC

GTTGCTAGCCGTCAGGACGAAGACTAAAAGCTTCAGGC

SEQ ID NO: 71

NAME: gi|438610|gb|AAC37875.1| integrase, partial [Human immunodeficiency virus 1]

SEQUENCE:
FLDGIDKAQEEHEKYHSNWRAMASDFNLPPVVAKEIVASCDKCQLKGEAMHGQ
VDCSPGIWQLDCTHLEG
KVILVAVHVASGYIEAEVIPAETGQETAYFLLKLAGRWPVKTVHTDNGSNFTSTT
VKAACWWAGIKQEFG
IPYNPQSQGVIESMNKELKKIIGQVRDQAEHLKTAVQMAVFIHNFKRKGGIGGYS
AGERIVDIIATDIQT
KELQKQITKIQNFRVYYRDSRDPVWKGPAKLLWKGEGAVVIQDNSDIKVVPRRK
AKIIRDYGKQMAGDDC
VASRQDED

SEQ ID NO: 72

NAME: gi|545612232|ref]WP_021736722.1| type V CRISPR-associated protein Cpf1

[Acidaminococcus sp. BV3L6]

SEQUENCE:

MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELKPIIDRI
YKTYADQCLQLVQ
LDWENLSAAIDSYRKEKTEETRNALIEEQATYRNAIHDYFIGRTDNLTDAINKRH
AEIYKGLFKAELFNG
KVLKQLGTVTTTEHENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQD
NFPKFKENCHIFTR
LITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYNQLLGGISRE
AGTEKIKGLNEV
LNLAIQKNDETAHIIASLPHRFIPLFKQILSDRNTLSFILEEFKSDEEVIQSFCKYKTL
LRNENVLETAE
ALFNELNSIDLTHIFISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKE
KVQRSLKHEDINL
QEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILKSQLDSLLGL
YHLLDWFAVDESN

EVDPEFSARLTGIKLEMEPSLSFYNKARNYATKKPYSVEKFKLNFQMPTLASGW
DVNKEKNNGAILFVKN
GLYYLGIMPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKA
VTAHFQTHTTPILLSN
NFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKGYREALCKWIDFTRDFLS
KYTKTTSIDLSSLRP
SSQYKDLGEYYAELNPLLYHISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHH
GKPNLHTLYWTGLFS
PENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPIPDTLYQ
ELYDYVNHRLSHDLSD
EARALLPNVITKEVSHEIIKDRRFTSDKFFFHVPITLNYQAANSPSKFNQRVNAYL
KEHPETPIIGIDRG
ERNLIYITVIDSTGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIK
DLKQGYLSQVIHEIV
DLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLIDKLNCLVLKDYPAE
KVGGVLNPYQLTDQFT
SFAKMGTQSGFLFYVPAPYTSKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLH
YDVKTGDFILHFKMN
RNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTGRYRD
LYPANELIALLEEKG
IVFRDGSNILPKLLENDDSHAIDTMVALIRSVLQMRNSNAATGEDYINSPVRDLN
GVCFDSRFQNPEWPM
DADANGAYHIALKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRN

SEQ ID NO: 73

NAME: gi|769142322|ref|WP_044919442.1| type V CRISPR-associated protein Cpf1

[Lachnospiraceae bacterium MA2020]

SEQUENCE:
MYYESLTKQYPVSKTIRNELIPIGKTLDNIRQNNILESDVKRKQNYEHVKGILDEY
HKQLINEALDNCTL
PSLKIAAEIYLKNQKEVSDREDFNKTQDLLRKEVVEKLKAHENFTKIGKKDILDL
LEKLPSISEDDYNAL

ESFRNFYTYFTSYNKVRENLYSDKEKSSTVAYRLINENFPKFLDNVKSYRFVKTA
GILADGLGEEEQDSL
FIVETFNKTLTQDGIDTYNSQVGKINSSINLYNQKNQKANGFRKIPKMKMLYKQI
LSDREESFIDEFQSD
EVLIDNVESYGSVLIESLKSSKVSAFFDALRESKGKNVYVKNDLAKTAMSNIVFE
NWRTFDDLLNQEYDL
ANENKKKDDKYFEKRQKELKKNKSYSLEHLCNLSEDSCNLIENYIHQISDDIENII
NNETFLRIVINEH
DRSRKLAKNRKAVKAIKDFLDSIKVLERELKLINSSGQELEKDLIVYSAHEELLVE
LKQVDSLYNMTRNY
LTKKPFSTEKVKLNFNRSTLLNGWDRNKETDNLGVLLLKDGKYYLGIMNTSAN
KAFVNPPVAKTEKVFKK
VDYKLLPVPNQMLPKVFFAKSNIDFYNPSSEIYSNYKKGTHKKGNMFSLEDCHN
LIDFFKESISKHEDWS
KFGFKFSDTASYNDISEFYREVEKQGYKLTYTDIDETYINDLIERNELYLFQIYNK
DFSMYSKGKLNLHT
LYFMMLFDQRNIDDVVYKLNGEAEVFYRPASISEDELIIHKAGEEIKNKNPNRAR
TKETSTFSYDIVKDK
RYSKDKFTLHIPITMNFGVDEVKRFNDAVNSAIRIDENVNVIGIDRGERNLLYVV
VIDSKGNILEQISLN
SIINKEYDIETDYHALLDEREGGRDKARKDWNTVENIRDLKAGYLSQVVNVVAK
LVLKYNAIICLEDLNF
GFKRGRQKVEKQVYQKFEKMLIDKLNYLVIDKSREQTSPKELGGALNALQLTSK
FKSFKELGKQSGVIYY
VPAYLTSKIDPTTGFANLFYMKCENVEKSKRFFDGFDFIRFNALENVFEFGFDYR
SFTQRACGINSKWTV
CTNGERIIKYRNPDKNNMFDEKVVVVTDEMKNLFEQYKIPYEDGRNVKDMIISN
EEAEFYRRLYRLLQQT
LQMRNSTSDGTRDYIISPVKNKREAYFNSELSDGSVPKDADANGAYNIARKGLW
VLEQIRQKSEGEKINL
AMTNAEWLEYAQTHLL

SEQ ID NO: 74

NAME: gi|489130501|ref|WP_003040289.11 type V CRISPR-associated protein Cpf1

[Francisella tularensis]

SEQUENCE:

MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAKQIIDK
YHQFFIEEILSSVC
ISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKDTIKKQISEYIKDSEKFKNLFNQ
NLIDAKKGQESDLIL
WLKQSKDNGIELFKANSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIP
TSIIYRIVDDNLPK
FLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFSLDEVFEI
ANFNNYLNQSGITK
FNTIIGGKFVNGENTKRKGINEYINLYSQQINDKTLKKYKMSVLFKQILSDTESKS
FVIDKLEDDSDVVT
TMQSFYEQIAAFKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQV
FDDYSVIGTAVLEY
ITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFNKHRDIDKQCRFE
EILANFAAIPMIFD
EIAQNKDNLAQISIKYQNQGKKDLLQASAEDDVKAIKDLLDQTNNLLHKLKIFHI
SQSEDKANILDKDEH
FYLVFEECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEP
DNTAILFIKDDKYYL
GVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGANKMLPKVFFSAKSIKFYNP
SEDILRIRNHSTHTKN
GSPQKGYEKFEFNIEDCRKFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYRE
VENQGYKLTFENIS
ESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQDVVYKL
NGEAELFYRKQSIPKK
ITHPAKEAIANKNKDNPKKESVFEYDLIKDKRFTEDKFFFHCPITINFKSSGANKF
NDEINLLLKEKAND

VHILSIDRGERHLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRD
SARKDWKKINNIKEM
KEGYLSQVVHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVEKQVYQKLEKMLIEKL
NYLVFKDNEFDKTGG
VLRAYQLTAPFETFKKMGKQTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKS
QEFFSKFDKICYNLD
KGYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYPTKEL

EKLLKDYSIEYGHGEC
IKAAICGESDKKFFAKLTSVLNTILQMRNSKTGTELDYLISPVADVNGNFFDSRQ
APKNMPQDADANGAY
HIGLKGLMLLGRIKNNQEGKKLNLVIKNEEYFEFVQNRNN

SEQ ID NO: 75

NAME: gi|502240446|ref|WP_012739647.1| type V CRISPR-associated protein Cpfl

[[Eubacterium] eligens]

SEQUENCE:
MNGNRSIVYREFVGVIPVAKTLRNELRPVGHTQEHIIQNGLIQEDELRQEKSTELK
NIMDDYYREYIDKS
LSGVTDLDFTLLFELMNLVQSSPSKDNKKALEKEQSKMREQICTHLQSDSNYKNI
FNAKLLKEILPDFIK
NYNQYDVKDKAGKLETLALFNGFSTYFTDFFEKRKNVFTKEAVSTSIAYRIVHE
NSLIFLANMTSYKKIS
EKALDEIEVIEKNNQDKMGDWELNQIFNPDFYNMVLIQSGIDFYNEICGVVNAH
MNLYCQQTKNNYNLFK
MRKLHKQILAYTSTSFEVPKMFEDDMSVYNAVNAFIDETEKGNIIGKLKDIVNKY
DELDEKRIYISKDFY
ETLSCFMSGNWNLITGCVENFYDENIHAKGKSKEEKVKKAVKEDKYKSINDVND
LVEKYIDEKERNEFKN
SNAKQYIREISNIITDTETAHLEYDDHISLIESEEKADEMKKRLDMYMNMYHWA
KAFIVDEVLDRDEMFY
SDIDDIYNILENIVPLYNRVRNYVTQKPYNSKKIKLNFQSPTLANGWSQSKEFDN
NAIILIRDNKYYLAI

FNAKNKPDKKIIQGNSDKKNDNDYKKMVYNLLPGANKMLPKVFLSKKGIETFK
PSDYIISGYNAHKHIKT
SENFDISFCRDLIDYFKNSIEKHAEWRKYEFKFSATDSYSDISEFYREVEMQGYRI
DWTYISEADINKLD
EEGKIYLFQIYNKDFAENSTGKENLHTMYFKNIFSEENLKDIIIKLNGQAELFYRR
ASVKNPVKHKKDSV
LVNKTYKNQLDNGDVVRIPIPDDIYNEIYKMYNGYIKESDLSEAAKEYLDKVEV
RTAQKDIVKDYRYTVD
KYFIHTPITINYKVTARNNVNDMVVKYIAQNDDIHVIGIDRGERNLIYISVIDSHG
NIVKQKSYNILNNY
DYKKKLVEKEKTREYARKNWKSIGNIKELKEGYISGVVHEIAMLIVEYNAIIAME
DLNYGFKRGRFKVER
QVYQKFESMLINKLNYFASKEKSVDEPGGLLKGYQLTYVPDNIKNLGKQCGVIF
YVPAAFTSKIDPSTGF

ISAFNFKSISTNASRKQFFMQFDEIRYCAEKDMFSFGFDYNNFDTYNITMGKTQW
TVYTNGERLQSEFNN
ARRTGKTKSINLTETIKLLLEDNEINYADGHDIRIDMEKMDEDKKSEFFAQLLSLY
KLTVQMRNSYTEAE
EQENGISYDKIISPVINDEGEFFDSDNYKESDDKECKMPKDADANGAYCIALKGL
YEVLKIKSEWTEDGF
DRNCLKLPHAEWLDFIQNKRYE

SEQ ID NO: 76

NAME: gi|537834683|ref|WP_020988726.1| type V CRISPR-associated protein Cpf1

[Leptospira inadai]

SEQUENCE:
MEDYSGFVNIYSIQKTLRFELKPVGKTLEHIEKKGFLKKDKIRAEDYKAVKKIIDK
YHRAYIEEVFDSVL
HQKKKKDKTRFSTQFIKEIKEFSELYYKTEKNIPDKERLEALSEKLRKMLVGAFK
GEFSEEVAEKYKNLF
SKELIRNEIEKFCETDEERKQVSNFKSFTTYFTGFHSNRQNIYSDEKKSTAIGYRII
HQNLPKFLDNLKI

IESIQRRFKDFPWSDLKKNLKKIDKNIKLTEYFSIDGFVNVLNQKGIDAYNTILGG
KSEESGEKIQGLNE
YINLYRQKNNIDRKNLPNVKILFKQILGDRETKSFIPEAFPDDQSVLNSITEFAKYL
KLDKKKKSIIAEL
KKFLSSFNRYELDGIYLANDNSLASISTFLFDDWSFIKKSVSFKYDESVGDPKKKI
KSPLKYEKEKEKWL
KQKYYTISFLNDAIESYSKSQDEKRVKIRLEAYFAEFKSKDDAKKQFDLLERIEEA
YAIVEPLLGAEYPR
DRNLKADKKEVGKIKDFLDSIKSLQFFLKPLLSAEIFDEKDLGFYNQLEGYYEEID
SIGHLYNKVRNYLT
GKIYSKEKFKLNFENSTLLKGWDENREVANLCVIFREDQKYYLGVMDKENNTIL
SDIPKVKPNELFYEKM
VYKLIPTPHMQLPRIIFSSDNLSIYNPSKSILKIREAKSFKEGKNFKLKDCHKFIDFY
KESISKNEDWSR
FDFKFSKTSSYENISEFYREVERQGYNLDFKKVSKFYIDSLVEDGKLYLFQIYNKD
FSIFSKGKPNLHTI
YFRSLFSKENLKDVCLKLNGEAEMFFRKKSINYDEKKKREGHHPELFEKLKYPIL
KDKRYSEDKFQFHLP
ISLNFKSKERLNFNLKVNEFLKRNKDINIIGIDRGERNLLYLVMINQKGEILKQTLL
DSMQSGKGRPEIN

YKEKLQEKEIERDKARKSWGTVENIKELKEGYLSIVIHQISKLMVENNAIVVLED
LNIGFKRGRQKVERQ
VYQKFEKMLIDKLNFLVFKENKPTEPGGVLKAYQLTDEFQSFEKLSKQTGFLFY
VPSWNTSKIDPRTGFI
DFLHPAYENIEKAKQWINKFDSIRFNSKMDWFEFTADTRKFSENLMLGKNRVW
VICTTNVERYFTSKTAN
SSIQYNSIQITEKLKELFVDIPFSNGQDLKPEILRKNDAVFFKSLLFYIKTTLSLRQN
NGKKGEEEKDFI
LSPVVDSKGRFFNSLEASDDEPKDADANGAYHIALKGLMNLLVLNETKEENLSR
PKWKIKNKDWLEFVWE
RNR

SEQ ID NO: 77

NAME: gi|739008549|ref|WP_036890108.1| type V CRISPR-associated protein Cpf1

[Porphyromonas crevioricanis]

SEQUENCE:
MDSLKDFTNLYPVSKTLRFELKPVGKTLENIEKAGILKEDEHRAESYRRVKKIIDT
YHKVFIDSSLENMA
KMGIENEIKAMLQSFCELYKKDHRTEGEDKALDKIRAVLRGLIVGAFTGVCGRR
ENTVQNEKYESLFKEK
LIKEILPDFVLSTEAESLPFSVEEATRSLKEFDSFTSYFAGFYENRKNIYSTKPQSTA
IAYRLIHENLPK
FIDNILVFQKIKEPIAKELEHIRADFSAGGYIKKDERLEDIFSLNYYIHVLSQAGIEK
YNALIGKIVTEG
DGEMKGLNEHINLYNQQRGREDRLPLFRPLYKQILSDREQLSYLPESFEKDEELL
RALKEFYDHIAEDIL
GRTQQLMTSISEYDLSRIYVRNDSQLTDISKKMLGDWNAIYMARERAYDHEQAP
KRITAKYERDRIKALK
GEESISLANLNSCIAFLDNVRDCRVDTYLSTLGQKEGPHGLSNLVENVFASYHEA
EQLLSFPYPEENNLI
QDKDNVVLIKNLLDNISDLQRFLKPLWGMGDEPDKDERFYGEYNYIRGALDQVI
PLYNKVRNYLTRKPYS
TRKVKLNFGNSQLLSGWDRNKEKDNSCVILRKGQNFYLAIMNNRHKRSFENKM
LPEYKEGEPYFEKMDYK
FLPDPNKMLPKVFLSKKGIEIYKPSPKLLEQYGHGTHKKGDTFSMDDLHELIDFF
KHSIEAHEDWKQFGF
KFSDTATYENVSSFYREVEDQGYKLSFRKVSESYVYSLIDQGKLYLFQIYNKDFS
PCSKGTPNLHTLYWR
MLFDERNLADVIYKLDGKAEIFFREKSLKNDHPTHPAGKPIKKKSRQKKGEESLF

EYDLVKDRRYTMDKF
QFHVPITMNFKCSAGSKVNDMVNAHIREAKDMHVIGIDRGERNLLYICVIDSRGT
ILDQISLNTINDIDY

HDLLESRDKDRQQEHRNWQTIEGIKELKQGYLSQAVHRIAELMVAYKAVVALE
DLNMGFKRGRQKVESSV
YQQFEKQLIDKLNYLVDKKKRPEDIGGLLRAYQFTAPFKSFKEMGKQNGFLFYIP
AWNTSNIDPTTGFVN
LFHVQYENVDKAKSFFQKFDSISYNPKKDWFEFAFDYKNFTKKAEGSRSMWILC
THGSRIKNFRNSQKNG
QWDSEEFALTEAFKSLFVRYEIDYTADLKTAIVDEKQKDFFVDLLKLFKLTVQM
RNSWKEKDLDYLISPV
AGADGRFFDTREGNKSLPKDADANGAYNIALKGLWALRQIRQTSEGGKLKLAIS
NKEWLQFVQERSYEKD

SEQ ID NO: 78

NAME: gi|517171043|ref|WP_018359861.1| type V CRISPR-associated protein Cpf1

[Porphyromonas macacae]

SEQUENCE:
MKTQHFFEDFTSLYSLSKTIRFELKPIGKTLENIKKNGLIRRDEQRLDDYEKLKKV
IDEYHEDFIANILS
SFSFSEEILQSYIQNLSESEARAKIEKTMRDTLAKAFSEDERYKSIFKKELVKKDIP
VWCPAYKSLCKKF
DNFTTSLVPFHENRKNLYTSNEITASIPYRIVHVNLPKFIQNIEALCELQKKMGAD
LYLEMMENLRNVWP
SFVKTPDDLCNLKTYNHLMVQSSISEYNRFVGGYSTEDGTKHQGINEWINIYRQR
NKEMRLPGLVFLHKQ
ILAKVDSSSFISDTLENDDQVFCVLRQFRKLFWNTVSSKEDDAASLKDLFCGLSG
YDPEAIYVSDAHLAT
ISKNIFDRWNYISDAIRRKTEVLMPRKKESVERYAEKISKQIKKRQSYSLAELDDL
LAHYSEESLPAGFS
LLSYFTSLGGQKYLVSDGEVILYEEGSNIWDEVLIAFRDLQVILDKDFTEKKLGK
DEEAVSVIKKALDSA
LRLRKFFDLLSGTGAEIRRDSSFYALYTDRMDKLKGLLKMYDKVRNYLTKKPYS
IEKFKLHFDNPSLLSG

WDKNKELNNLSVIFRQNGYYYLGIMTPKGKNLFKTLPKLGAEEMFYEKMEYKQ
IAEPMLMLPKVFFPKKT
KPAFAPDQSVVDIYNKKTFKTGQKGFNKKDLYRLIDFYKEALTVHEWKLFNFSF
SPTEQYRNIGEFFDEV

REQAYKVSMVNVPASYIDEAVENGKLYLFQIYNKDFSPYSKGIPNLHTLYWKAL
FSEQNQSRVYKLCGGG
ELFYRKASLHMQDTTVHPKGISIHKKNLNKKGETSLFNYDLVKDKRFTEDKFFF
HVPISINYKNKKITNV
NQMVRDYIAQNDDLQIIGIDRGERNLLYISRIDTRGNLLEQFSLNVIESDKGDLRT
DYQKILGDREQERL
RRRQEWKSIESIKDLKDGYMSQVVHKICNMVVEHKAIVVLENLNLSFMKGRKK
VEKSVYEKFERMLVDKL
NYLVVDKKNLSNEPGGLYAAYQLTNPLFSFEELHRYPQSGILFFVDPWNTSLTDP
STGFVNLLGRINYTN
VGDARKFFDRFNAIRYDGKGNILFDLDLSRFDVRVETQRKLWTLTTFGSRIAKSK
KSGKWMVERIENLSL
CFLELFEQFNIGYRVEKDLKKAILSQDRKEFYVRLIYLFNLMMQIRNSDGEEDYIL
SPALNEKNLQFDSR
LIEAKDLPVDADANGAYNVARKGLMVVQRIKRGDHESIHRIGRAQWLRYVQEGI
VE

SEQ ID NO: 79

NAME: Integrase protein sequence found on the Uniprot site. DNA sequence was obtained from GenBank.

SEQUENCE:
TTTTTAGATGGAATAGATAAGGCCCAAGATGAACATGAGAAATATCACAGTA
ATTGGAGAGCAATGGCTAGTGATTTTAACCTGCCACCTGTAGTAGCAAAAGA
AATAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGAGAAGCCATGCATGGA
CAAGTAGACTGTAGTCCAGGAATATGGCAACTAGATTGTACACATTTAGAAG
GAAAAGTTATCCTGGTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGA
AGTTATTCCAGCAGAAACAGGGCAGGAAACAGCATATTTTCTTTTAAAATTA
GCAGGAAGATGGCCAGTAAAAACAATACATACTGACAATGGCAGCAATTTCA

CCGGTGCTACGGTTAGGGCCGCCTGTTGGTGGGCGGGAATCAAGCAGGAATT
TGGAATTCCCTACAATCCCCAAAGTCAAGGAGTAGTAGAATCTATGAATAAA
GAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAG
ACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGA
TTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACA
TACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGGGT
TTATTACAGGGACAGCAGAAATCCACTTTGGAAAGGACCAGCAAAGCTCCTC
TGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGTA
GTGCCAAGAAGAAAAGCAAAGATCATTAGGGATTATGGAAAACAGATGGCA
GGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAG

SEQ ID NO: 80

NAME: sp|P04585|1148-1435

SEQUENCE:
FLDGIDKAQDEHEKYHSNWRAMASDFNLPPVVAKEIVASCDKCQLKGEAMHGQ
VDCSPGIWQLDCTHLEGKVILVAVHVASGYIEAEVIPAETGQETAYFLLKLAGR
WPVKTIHTDNGSNFTGATVRAACWWAGIKQEFGIPYNPQSQGVVESMNKELKKI
IGQVRDQAEHLKTAVQMAVFIHNFKRKGGIGGYSAGERIVDIIATDIQTKELQKQI
TKIQNFRVYYRDSRNPLWKGPAKLLWKGEGAVVIQDNSDIKVVPRRKAKIIRDY
GKQMAGDDCVASRQDED

SEQ ID NO: 81

a protein domain that characterizes zinc finger proteins

CX(2-4)CX(12)HX(3-5)H (X(2-4) means XX or XXX or XXXX for example)

SEQ ID NO: 82
>gi|1616606|emb|X97044.1| Mouse mammary tumor virus 5' LTR DNA
ATGCCGCGCCTGCAGCAGAAATGGTTGAACTCCCGAGAGTGTCCTACACTTA
GGGGAGAAGCAGCCAAGG
GGTTGTTTCCCACCCAGAACGACCCATCTGCGCACACACGGATGAGCCCGTC
AAACAAAGACATATTCAT
TCTCTGCTGCAAACTTGGCATAGCTCTGCTTTGCCTGGGGCTATTGGGGGAAG
TTGCGGTTCATGCTCGC

AGGGCTCTCACCCTTGACTCTTTTAATAGCTCTTCTGTGCAAGATTACAATCT
AAACAATTCGGAGAACT
CGACCTTCCTCCTGAGGCAAGGACCACAGCCAACTTCCTCTTACAAGCCGCA
TCGATTTAGTCCTTCAGA
AATAGAAATAAGAATGCTTGCTAAAAATTATATTTTTACCAATGAGACCAAT
CCAATAGGTCGATTATTA
ATTACTATGTTAAGAAATGAATCATTATCTTTTAGTACTATTTTTACTCAAATT
CAGAAGTTAGAAATGG
GAATAGAAAATAGAAAGAGACGCTCAGCCTCAGTTGAAGAACAGGTGCAAG
GACTAAGGGCCTCAGGCCT
AGAAGTAAAAAGGGGGAAGAGGAGTGCGCTTGTCAAAATAGGAGACAGGTG
GTGGCAACCAGGAACTTAT
AGGGGACCTTACATCTACAGACCAACAGACGCCCCCTTACCGTATACAGGAA
GATATGACCTAAATTTTG
ATAGGTGGGTCACAGTCAATGGCTATAAAGTGTTATACAGATCCCTCCCCTTT
CGTGAAAGGCTCGCCAG
AGCTAGACCTCCTTGGTGCGTGTTGTCTCAGGAAGAAAAAGACGACATGAAA

CAACAGGTACATGATTAT

ATTTATCTAGGAACAGGAATGAACTTTTGGAGATATTATACCAAGGAGGGGG

CAGTGGCTAGACTATTAG

AACACATTTCTGCAGATACTAATAGCATGAGTTATTATGATTAGCCTTTATTG

GCCCAATCTTGTGGTTC

CCAGGGTTCAAGTAGGTTCATGGTCACAAACTGTTCTTAAAAACAAGGATGT

GAGACAAGTGGTTTCCTG

GCTTGGTTTGGTATCAAATGTTTTGATCTGAGCTCTGAGTGTTCTGTTTTCCTA

TGTTCTTTTGGAATCT

ATCCAAGTCTTATGTAAATGCTTATGTAAACCAAAGTATAAAAGAGTGCTGA

TTTTTTGAGTAAACTTGC

AACAGTCCTAACATTCACCTCTCGTGTGTTTGTGTCTGTTCGCCATCCCGTCTC

CGCTCGTCACTTATCC

TTCACTTTCCAGAGGGTCCCCCCGCAGACCCCGGTGACCCTCAGGTTGGCCG

ACTGCGGCA

SEQ ID NO: 83

>gi|1403387|emb|X98457.1| Mouse mammary tumor virus 3' LTR

ATGCCGCGCCTGCAGCAGAAATGGTTGAACTCCCGAGAGTGTCCTACACTTA

GGAGAGAAGCAGCCAAGG

GGTTGTTTCCCACCAAGGACGACCCGTCTGCGTGCACGCGGATGAGCCCATC

AGACAAAGACATACTCAT

TCTCTGCTGCAAACTTGGCATAGCTCTGCTTTGCCTGGGGCTATTGGGGGAAG

TTGCGGTTCGTGCTCGC

AGGGCTCTCACCCTTGATTCTTTTAATAACTCTTCTGTGCAAGATTACAATCT

AAACGATTCGGAGAACT

CGACCTTCCTCCTGGGGCAAGGACCACAGCCAACTTCCTCTTACAAGCCACA

CCGACTTTGTCCTTCAGA

AATAGAAATAAGAATGCTTGCTAAAAATTATATTTTTACCAATGAGACCAAT

CCAATAGGTCGATTATTA

ATCATGATGTTTAGAAATGAATCTTTGTCTTTTAGCACTATATTTACTCAAATT

CAAAGGTTAGAAATGG

GAATAGAAAATAGAAAGAGACGCTCAACCTCAGTTGAAGAACAGGTGCAAG

GACTAAGGGCCTCAGGCCT

AGAAGTAAAAAGGGGAAAGAGGAGTGCGCTTGTCAAAATAGGAGACAGGTG

GTGGCAACCAGGGACTTAT

AGGGGACCTTACATCTACAGACCAACAGACGCCCCGCTACCATATACAGGAA

GATACGATTTAAATTTTG

ATAGGTGGGTCACAGTCAACGGCTATAAAGTGTTATACAGATCCCTCCCCCTT

CGTGAAAGACTCGCCAG

GGCTAGACCTCCTTGGTGTGTGTTAACTCAGGAAGAAAAAGACGACATGAAA
CAACAGGTACATGATTAT
ATTTATCTAGGAACAGGAATGAACTTCTGGGGAAAGATATTTGACTACACCG
AAGAGGGAGCTATAGCAA

AAATTATATATAATATGAAATATACTCATGGGGGTCGCATTGGCTTCGATCCC
TTTTGAAACATTTATAA
ATACAATTAGGTCTACCTTGCGGTTCCCAAGGTTTAAGTAAGTTCAGGGTCAC
AAACTGTTCTTAAAACA
AGGATGTGAGACAAGTGGTTTCCTGACTTGGT

SEQ ID NO: 84

>gi|119662099|emb|AM076881.1| Human immunodeficiency virus 1 proviral 5' LTR, TAR element and U3, U5 and R repeat regions, clone PG232.14
GGCAAGAAATCCTTGATTTGTGGGTCTACTACACACAAGGCTTCTTCCCTGAT
TGGCAAAACTACACACC
GGGACCAGGGGTCAGATATCCACTGACCTTTGGATGGTGCTACAAGCTAGTG
CCAGTTGACCCAAAGGAA
GTAGAAGAGGCTAACCAAAGAGAAGACAACTGTTTGCTACACCCTATGAGCC
TGCATGGAATAGAGGACG
AAGACAGAGAAGTATTAAAGTGGCAGTTTGACAGCAGCCTAGCACGCAGAC
ACATGGCCCGCGAGCTACA
TCCAGAGTATTACAAAGACTGCTGACACAGAAAAGACTTTCCGCTAGGACTT
TCCACTGAGGCGTTCCAG
GGGGAGTGGTCTAGGCAGGACTAGGAGTGGCCAACCCTCAGATGCTGCATAT
AAGCAGCTGCTTTTCGCC
TGTACTAGGTCTCTCTAGGTGGACCAGATCTGAGCCTAGGCGCTCTCTGGCTA
TCTAAGGAACCCACTGC
TTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCTAAGTAGTGTGTGCCCGTCTG
TTGTGTGACTCTAGTAA
CTAGAGATCCCTCAGACCAACTTTAGTAGTGTAAAAAATCTCTAGCAGTGGC
GCCCGAACAGGGACCCGA
AAGTGAAAGCAGGACCAGAGGAGATCTCTCGACGCAGGACTCGGCTTGCTGA
AAGTGCACTCGGCAAGAG
GCGAGAGCAGCGGCGACTGGTGAGTACGCCGAATTTTATTTTGACTAGCGGA
GGCTAGAAGGAGAGAGAT
A

SEQ ID NO: 85

>gi|1072081|gb|U37267.1|HIV1U37267 Human immunodeficiency virus type 1 3' LTR region
ATGGGTGGCAAGTGGTCAGAAAGTAGTGTGGTTAGAAGGCATGTACCTTTAA
GACAAGGCAGCTATAGAT

CTTAGCCGCTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCAC

AGAGAAGATCAGTTGAAC

CAGAAGAAGATAGAAGAGGCCATGAAGAAGAAAACAACAGATTGTTCCGTT

TGTTCCGTTGGGGACTTTC

CAGGAGACGTGGCCTGAGTGATAAGCCGCTGGGGACTTTCCGAAGAGGCGTG

ACGGGACTTTCCAAGGCG

ACGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATGCTGCATATAA

GCAGCTGCTTTCTGCCTG

TACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAAC

TAGGGAACCCACTGCTT

AAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTT

GTGTGACTCTGGTATCT

AGA

THERE ARE NO SEQ ID NOS: 86-99

SEQ ID NO: 100

Oligo for insertion of neo into a cell's genome (using full sequences of 5' and 3' HIV LTRs

GACAAGACATCCTTGATTTGTGGGTCTATAACACACAAGGCTTCTTCCCTGAT

TGGCAAAACTACACACC

GGGACCAGGGACCAGATACCCACTGACCTTTGGATGGTGCTTCAAGCTAGTG

CCAGTTGACCCAAGGGAA

GTAGAAGAGGCCAATACAGGGGAAAACAACTGTTTGCTCCACCCTATGAGCC

AGCATGGAATGGAAGATG

ACCATAGAGAAGTATTAAAGTGGAAGTTTGACAGTATGCTAGCACGCAGACA

CCTGGCCCGCGAGCTACA

TCCGGAGTACTACAAAAACTGCTGACATGGAGGGACTTTCCGCTGGGACTTT

CCATTGGGGCGTTCCAGG

AGGTGTGGTCTGGGCGGGACAAGGGAGTGGTCAACCCTCAGATGCTGCATAT

AAGCAGCTGCTTTTCGCT

TGTACTGGGTCTCTTTAGGTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTA

CCTGAGGAACCCACTGC

TTAAGCCTCAATAAAGCTTGCCTTGAGTGCTCTAAGTAGTGTGTGCCCGTCTG

TTGTGTGACTCTGGTAA

CTAGAGATCCCTCAGACCCTTTTGGTAGTGTGGAAAATCTCTAGCAGATGATT

GAACAAGATGGATTGCAC

GCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCAC

AAC**ATGGGTGGCAAGTGGTCAG**

AAAGTAGTGTGGTTAGAAGGCATGTACCTTTAAGACAAGGCAGCTATAG

ATCTTAGCCGCTTTTTAAAAGAAAAG

GGGGGACTGGAAGGGCTAATTCACTCACAGAGAAGATCAGTTGAACCAG

AAGAAGATAGAAGAGGCCATGAAG

AAGAAAACAACAGATTGTTCCGTTTGTTCCGTTGGGGACTTTCCAGGAG

ACGTGGCCTGAGTGATAAGCCGCTGGG

GACTTTCCGAAGAGGCGTGACGGGACTTTCCAAGGCGACGTGGCCTGGG

CGGGACTGGGGAGTGGCGAGCCCTC

AGATGCTGCATATAAGCAGCTGCTTTCTGCCTGTACTGGGTCTCTCTGGT

TAGACCAGATCTGAGCCTGGGAGCTCT

CTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTG

AGTGCTTCAAGTAGTGTGTGCCCGTCTG

TTGTGTGACTCTGGTATCTAGA

First 5'LTR is underlined, plain text is neo, and 3'LTR is **bolded** (1179 bp)

SEQ ID NO: 101

An abbreviated version of 5'LTR and 3'LTR with neo sequence within (224 bp) First 5'LTR is underlined, plain text is neo, and 3'LTR is **bolded**

GACAAGACATCCTTGATTTGTGGGTCTATAACACACAAGGCTTCTTCCCTGAT

TGGCAAAACTACACACCATGATTGAACAAGATGGATTGCAC

GCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCAC

AAC**TTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCC**

CGTCTG

TTGTGTGACTCTGGTATCTAGA

Regarding SEQ ID NO: 72

Genbank Protein ID: WP_021736722.1

NCBI protein GI from NR Database or local GI (for proteins originated from WGS database): 545612232

Contig ID in WGS database: AWUR01000016.1

Contig description: Acidaminococcus sp. BV3L6 contig00028, whole genome shotgun sequence

Protein completeness: Complete

Proteins analyzed experimentally: 8

Non-redundant set: nr

Organism: Acidaminococcus_sp_BV3L6

Taxonomy:
Bacteria, Firmicutes,Negativicutes,Selenomonadales, Acidaminococcaceae, Acidaminococ

cus,Acidaminococcus sp. BV3L6

Regarding SEQ ID NO: 73

Genbank Protein ID: WP_044919442.1

NCBI protein GI from NR Database or local GI (for proteins originated from WGS database): 769142322

Contig ID in WGS database: JQKK01000008.1

Contig description: Lachnospiraceae bacterium MA2020

T348DRAFT_scaffold00007.7_C, whole genome shotgun sequence

Protein completeness: Complete

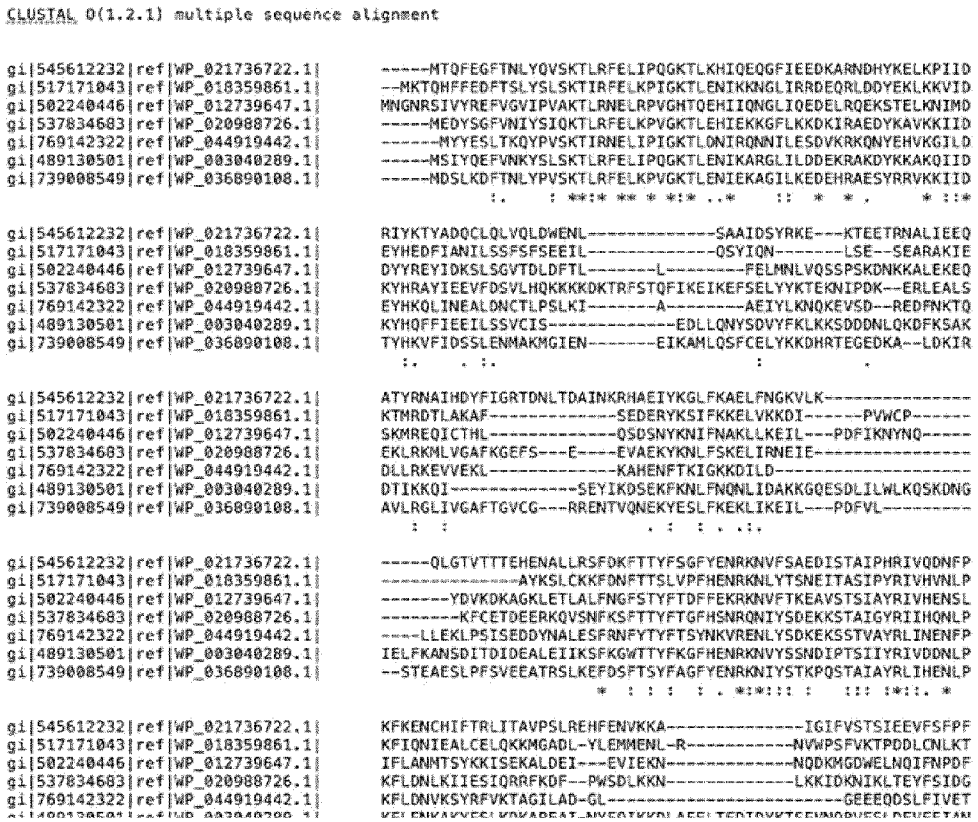
Proteins analyzed experimentally: 9

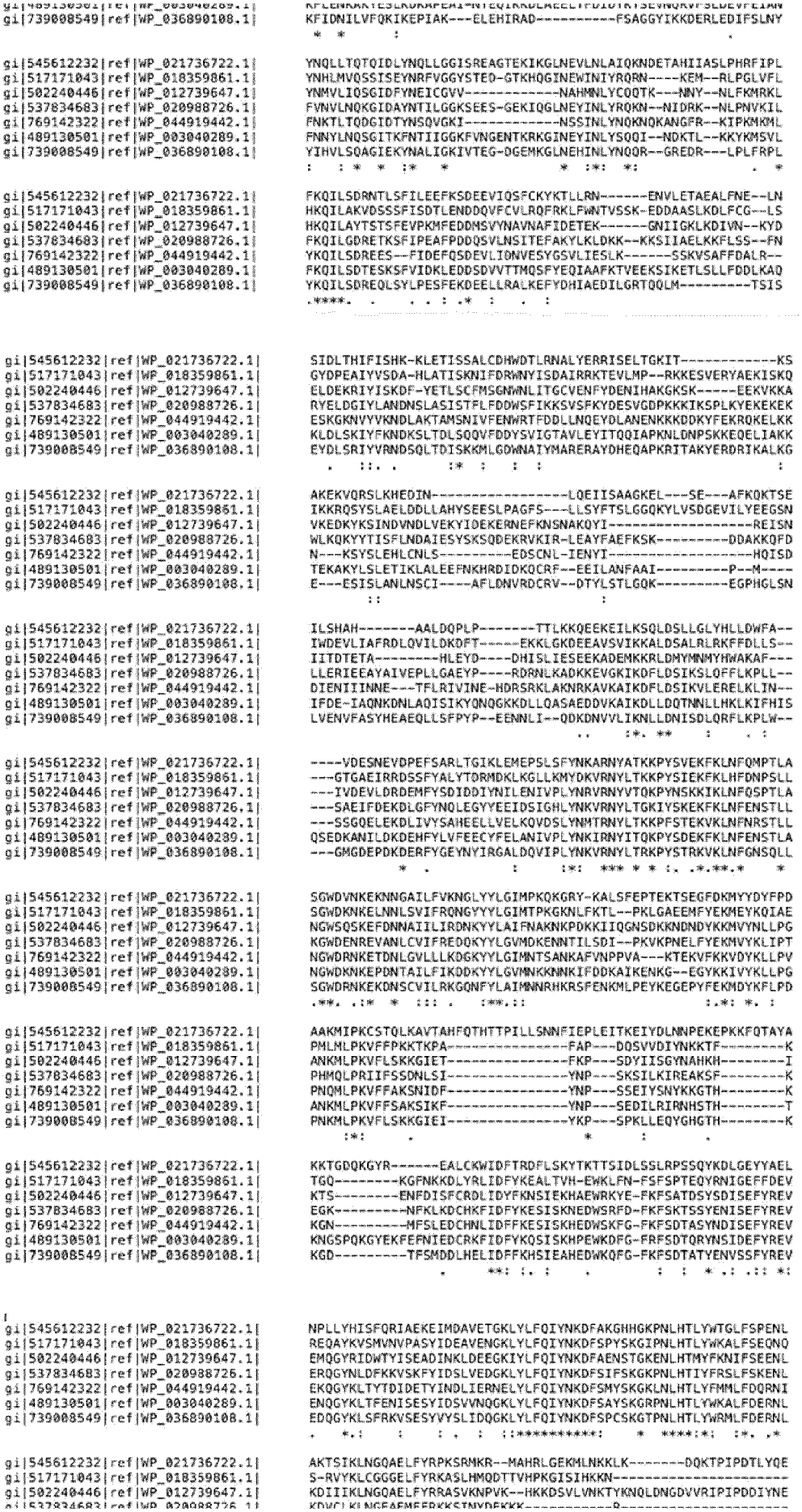
Non-redundant set: nr

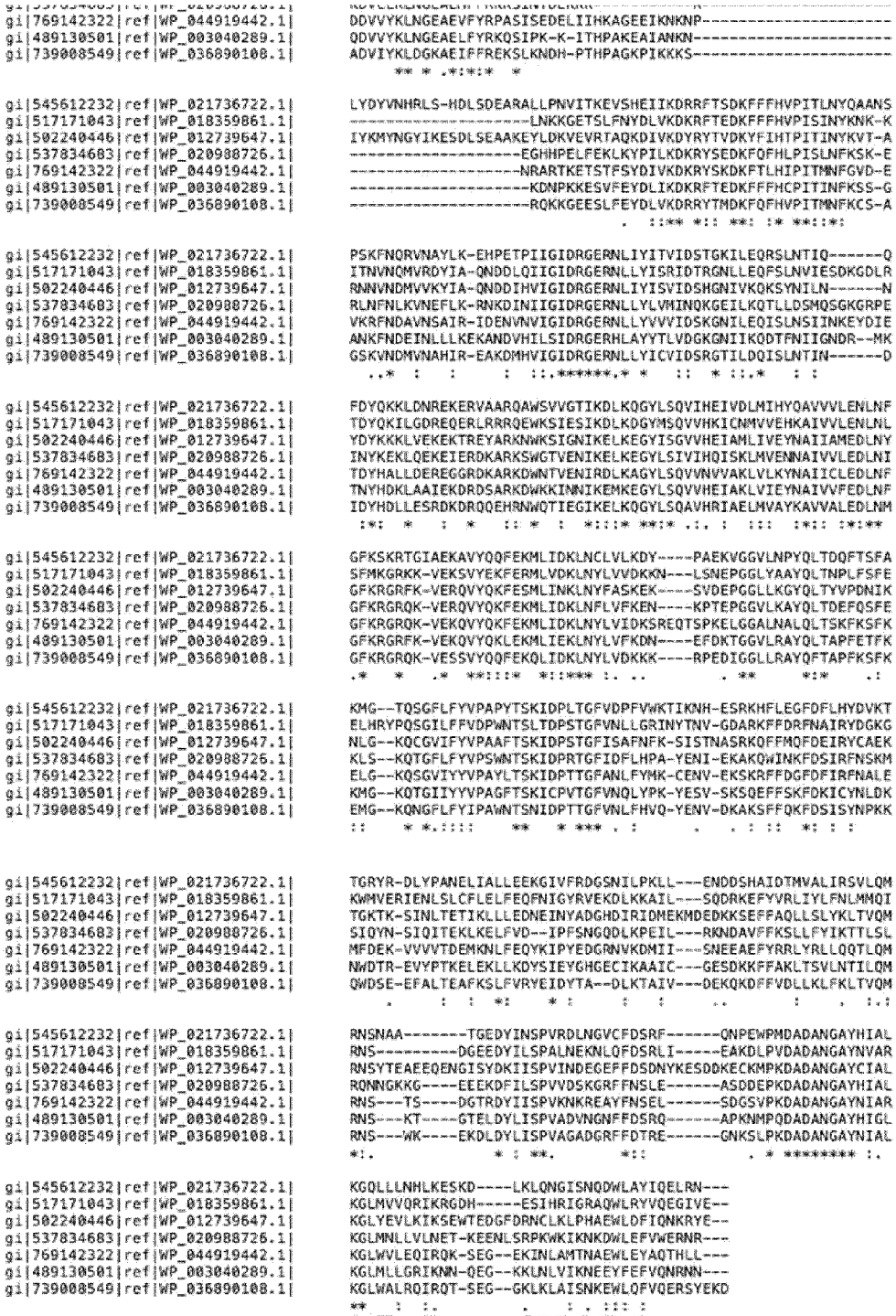
Organism: Lachnospiraceae_bacterium_MA2020

Taxonomy: Bacteria,Firmicutes,Clostridia,Clostridiales,Lachnospiraceae,unclassified Lachnospiraceae,Lachnospiraceae bacterium MA2020

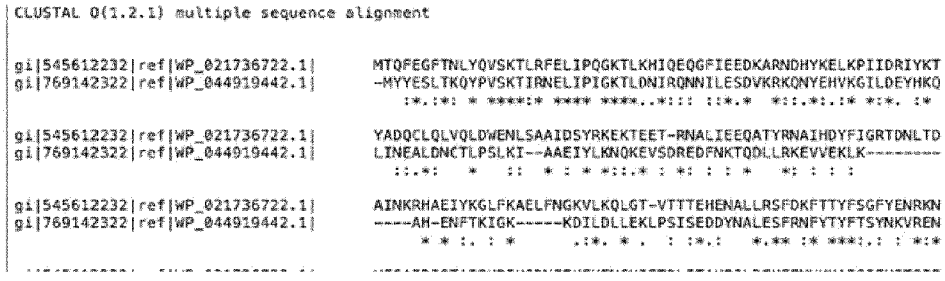
Additional nucleic acid sequences and protein sequences that can be used in the disclosed compositions and methods - CPF 1 alignment. SEQ ID NOS: 86-92; in order from the top to the bottom of the chart.

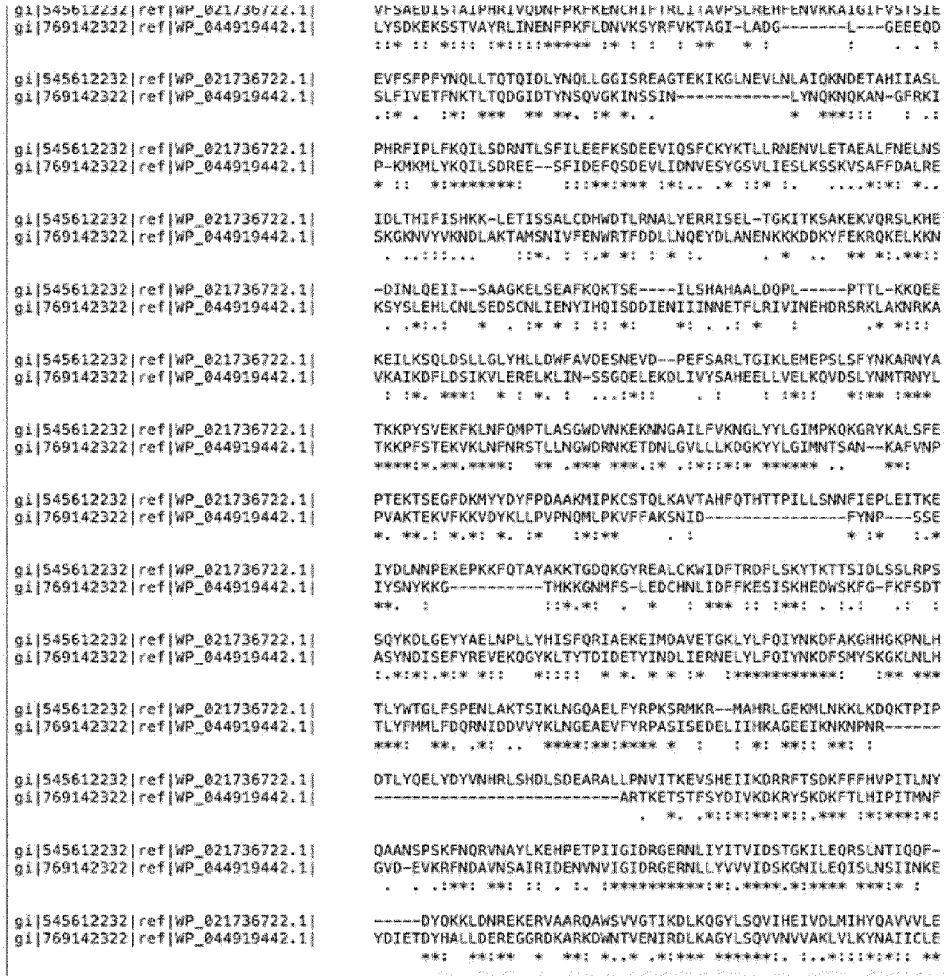






Additional nucleic acid sequences and protein sequences that can be used in the disclosed compositions and methods - Cfp1 human cleaving proteins alignment. SEQ ID NO: 86 (first row) and SEQ ID NO: 90 (second row).





Additional nucleic acid sequences and protein sequences that can be used in the disclosed compositions and methods. Table taken from Haft, D., et al. PLoS Computational Biology, November 2005, Vol. 1, Issue 6, pp. 474-483. SEQ ID NOS: 200-253; in order from the top to the bottom of the chart.

Table 1. Description of the Different *cas* Core Genes, CRISPR/Cas Subtypes, and the RAMP Module, Based on the New Cas Protein Families

Category	Gene	Example Locus	Specific HMM	COG	Putative Function/Family	Notes
Core protein	*cas1*	AF1870	TIGR00287	COG1518	Putative novel nuclease *	—
	cas2	AF1876	TIGR01573	COG1343, COG3512	—	—
		CT1518	TIGR01873	COG1343	—	Ecoli subtype-specific
	cas3	AF1874	TIGR01587	COG1203	Helicase (PF00271)	Core domain
		AF1875	TIGR03596	COG2254	Nuclease (PF01966)	HD domain
		YPO2467	TIGR02562	COG1203	Helicase (PF00271)	Ypest subtype-specific

	cas4	AF1877	TIGR00372	COG1468	RecB-family exonuclease [a]	—
	cas5	AF1872	TIGR02593	—	—	N-terminal domain
	cas6	AF1859	TIGR01877	COG1583	Possible RAMP [a]	When present, usually first
Ecoli subtype	cse1	CT1972	TIGR02547	—	—	—
	cse2	CT1973	TIGR02548	—	—	—
	cse3	CT1974	TIGR01907	—	—	—
	cse4	CT1975	TIGR01869	—	—	—
	cas5e	CT1976	TIGR01868	—	Cas5 N-terminal domain	—
Ypest subtype	csy1	YPO2465	TIGR02564	—	—	—
	csy2	YPO2464	TIGR02565	—	—	—
	csy3	YPO2463	TIGR02566	—	—	—
	csy4	YPO2462	TIGR02563	—	—	—
Nmeni subtype	csn1	SPs1176	TIGR01865	COG3513	HNH endonuclease?	—
	csn2	SPs1173	TIGR01866	—	—	Not always present
Dvulg subtype	csd1	CT1133	TIGR01863	—	—	—
	csd2	CT1132	TIGR02589	COG3649	—	—
	cas5d	CT1134	TIGR01876	—	Cas5 N-terminal domain	—
Tneap subtype	cst1	GTN1972	TIGR01908	—	Contains CXXC-CXXC motif	Occasionally absent
	cst2	GTN1971	TIGR02585	COG1857	Regulator (TIGR01875)	Related to Csa2
	cas5t	GTN1970	TIGR01895	COG1688	Cas5 N-terminal domain	—
Hmari subtype	csh1	TM1802	TIGR02591	—	Often contains CXXC-CXXC motif	—
	csh2	TM1801	TIGR02590	COG3649	Regulator (TIGR01875)	Related to Csd2
	cas5h	TM1800	TIGR02592	COG1688	Cas5 N-terminal domain	—
Apern subtype	csa1	AF1879	TIGR01896	COG4343	—	Usually proximal to repeat
	csa2	AF1871	TIGR02583	COG1857	Regulator (TIGR01875)	—
	csa3	AF1869	TIGR01884	COG0640	Helix-turn-helix, transcriptional regulator	Distantly related to PF03022
	csa4	MJ0385	TIGR01914	—	—	Occasionally absent
	csa5	AF1870	TIGR01878	—	—	Occasionally absent
	cas5a	AF1872	TIGR01874	COG1688	Cas5 N-terminal domain	—
Mtube subtype	csm1	TM1811	TIGR02578	COG1353	Putative novel polymerase [a]	Related to Cmr2
	csm2	TM1810	TIGR01870	COG1421	—	—
	csm3	TM1809	TIGR02582	COG1337	RAMP (PF03787)	Related to Cmr4
	csm4	TM1808	TIGR01903	COG1567	RAMP (PF08787)	—
	csm5	TM1807	TIGR01899	COG1332	RAMP (PF03787)	—
RAMP module	cmr1	TM1795	TIGR01894	COG1367	RAMP (PF03787)	—
	cmr2	TM1794	TIGR02577	COG1353	Putative novel polymerase [a]	Related to Csm1
	cmr3	TM1793	TIGR01888	COG1769	RAMP [a]	—
	cmr4	TM1792	TIGR02580	COG1336	RAMP (PF03787)	Related to Csm3
	cmr5	TM1791.1	TIGR01881	COG3337	—	—
	cmr6	TM1791	TIGR01898	COG1604	RAMP (PF03787)	—

Table 2. Other CRISPR/Cas Protein Families with No Identified Contextual Pattern

Gene Symbol	Example Locus	Specific HMM	COG	Putative Function	Subtypes Found in				
					Apern	Tneap	Mtube	RAMP	Other
csx1	MJ1666	TIGR01897	COG1517	Possible enzyme [a]	+	+	+	+	+
csx2	TM1812	TIGR02221	—	—		+	+	+	+
csx3	AF1864	TIGR02579	—	—	+	+		+	+
csx4	GSU0053	TIGR02570	—	—					+
csx5	GSU0054	TIGR02165	—	—					+
csx6	NE0113	TIGR02584	—	—				+	+
csx7	SSO1426	TIGR02581	COG1337	RAMP [a]					+

[a] Makarova et al. [14].

Editing target sequences and PAMs for Nrf2 (exon 2): Used for sgRNA design 1-3

SEQ ID NO: 254
GCGACGGAAAGAGTATGAGC TGG

SEQ ID NO: 255
TATTTGACTTCAGTCAGCGA CGG

SEQ ID NO: 256
TGGAGGCAAGATATAGATCT TGG
Primer Key for Detection of Integration at Nrf2 Target

Primer Set 1:

SEQ ID NO: 257
Primer 1:5'-GTGTTAATTTCAAACATCAGCAGC-3',

SEQ ID NO: 258
Primer 2: 5'- GACAAGACATCCTTGATTTG-3'

Primer Set 2:

SEQ ID NO: 259
Primer 1:5'-GAGGTTGACTGTGTAAATG-3',

SEQ ID NO: 260
Primer 2: 5'- GATACCAGAGTCACACAACAG-3'

Primer Set 3:

SEQ ID NO: 261
Primer 1: 5'-TCTACATTAATTCTCTTGTGC-3',

SEQ ID NO: 262
Primer 2:5'- GATACCAGAGTCACACAACAG-3'

Accession number for human CXCR4

Uniprot P61073

Ensembl gene ID: ENSG00000121966

Editing target sequence and PAM for CXCR4 (Exon 2): Used for sgRNA design1

SEQ ID NO: 263
GGGCAATGGATTGGTCATCC TGG
Primer Key for Detection of Integration at CXCR4 Target

Primer Set 1:

SEQ ID NO: 264
Primer 1: 5'- TCTACATTAATTCTCTTGTGC-3',

SEQ ID NO: 265
Primer 2: 5'- GACAAGACATCCTTGATTTG-3'

Primer Set 2:

SEQ ID NO: 266
Primer 1: 5'- TCTACATTAATTCTCTTGTGC-3',

SEQ ID NO: 267
Primer 2: 5'- GATACCAGAGTCACACAACAG -3'

Primer Set 3:

SEQ ID NO: 268
Primer 1: 5'- GAGGTTGACTGTGTAAATG -3',

SEQ ID NO: 269
Primer 2: 5'- GACAAGACATCCTTGATTTG-3'

Primer Set 4:

SEQ ID NO: 270
Primer 1: 5'- GAGGTTGACTGTGTAAATG -3',

SEQ ID NO: 271
Primer 2: 5'- GATACCAGAGTCACACAACAG -3'

Avi-tagged Cas9 for biotinylation

Sequence of the avi-tag used for Cas9 biotinylation

Amino acid seqeunce:
SEQ ID NO: 272
G G D L E G S G L N D I F E A Q K I E W H E *

Nucleic acid sequence:
SEQ ID NO: 273
GGCGGCGACCTCGAGGGTAGCGGTCTGAACGATATTTTTGAAGCGCAGAAAA
TTGAATGGCATGAATAA

REFERENCES CITED IN THE DESCRIPTION

Cited references

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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PATENTKRAV

1. System til indsætning af en donornukleinsyre i genomisk DNA, omfattende:

(a) et fusionsprotein, der omfatter:

i) et første protein omfattende et katalytisk inaktivt Cas9-protein;

ii) et andet protein omfattende en retroviral integrase og

iii) en linker, der bindet det første protein til det andet protein;

(b) et guide-RNA (gRNA) og

(c) en DNA-vektor, der omfatter: donor-DNA'et, en første retroviral, lang, terminal repeat (rLTR) og en anden rLTR,

hvor donor-DNA'et befinder sig mellem den første rLTR og anden rLTR.

2. System ifølge krav 1, hvor det første protein kodes for af et polynukleotid med mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, eller mindst 99 % sekvensidentitet med SEQ ID NO: 56; eller omfatter en aminosyresekvens med mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, eller mindst 99 % sekvensidentitet med SEQ ID NO: 52.

3. System ifølge krav 1 eller 2, hvor det første protein omfatter aminosyresekvensen ifølge SEQ ID NO: 52.

4. System ifølge et hvilket som helst af kravene 1-3, hvor det andet protein kodes for af et polynukleotid med mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, eller mindst 99 % sekvensidentitet ifølge et hvilket som helst af SEQ ID NO: 15, 17, 47, 62 eller 70; og/eller omfatter mindst 85 %, mindst 90 %, mindst 95 %, eller mindst 99 % sekvensidentitet ifølge et hvilket som helst af SEQ ID NO: 16, 18, 48, 63 eller 71.

5. System ifølge et hvilket som helst af kravene 1-4, hvor den retrovirale integrase er en HIV1-integrase eller en lentiviral integrase.

6. System ifølge et hvilket som helst af kravene 1-5, hvor den retrovirale integrase er en HIV1-integrase.

7. System ifølge et hvilket som helst af kravene 1-6, hvor det andet protein omfatter aminosyresekvensen ifølge SEQ ID NO: 71.

8. System ifølge et hvilket som helst af kravene 1-7, hvor linkeren er 4-8 aminosyrer i længden.

9. System ifølge et hvilket som helst af kravene 1-6, hvor gRNA'et er målrettet en mål-DNA-sekvens med en længde fra 16 til 24 basepar.

10. System ifølge et hvilket som helst af kravene 1-6, hvor den første eller anden rLTR omfatter en nukleotidsekvens valgt blandt SEQ ID NO: 59 og 60.

11. Fremgangsmåde *in vitro* til indsætning af en DNA-sekvens i genomisk DNA, omfattende:

a) identificering af en målsekvens i det genomiske DNA;

b) design af et fusionsprotein og guide-RNA (gRNA) som defineret i et hvilket som helst af kravene 1 til 10 til at binde til målsekvensen i det genomiske DNA;

c) design af en DNA-sekvens af interesse til at inkorporere i det genomiske DNA; og

d) tilvejebringelse af fusionsproteinet, gRNA'et og DNA-sekvensen af interesse til en celle ved teknikker, der muliggør indtrængen af fusionsproteinet, gRNA'et og DNA-sekvensen af interesse i cellen; hvor DNA-sekvensen af interesse integreres ved målsekvensen i det genomiske DNA.

12. Fremgangsmåde *in vitro* til hæmning af gentransskription i en celle, omfattende:

a) identificering af et ATG-startkodon i et gen;

b) design af et fusionsproteinsystem med et fusionsprotein som defineret i et hvilket som helst af kravene 1 til 10 og et guide-RNA (gRNA) til at binde til en målsekvens umiddelbart efter genets ATG-startkodon;

c) design af en DNA-sekvens af interesse, der er ét eller flere på hinanden efterfølgende stopkodoner; og

d) tilvejebringelse af fusionsproteinet, gRNA'et og DNA-sekvensen af interesse til en celle ved teknikker, der muliggør indtrængen af fusionsproteinet,

gRNA'et og DNA-sekvensen af interesse i cellen; hvor DNA-sekvensen af interesse integreres ved målsekvensen i det genomiske DNA; og hvor transskription af genet hæmmes.

DRAWINGS

Drawing

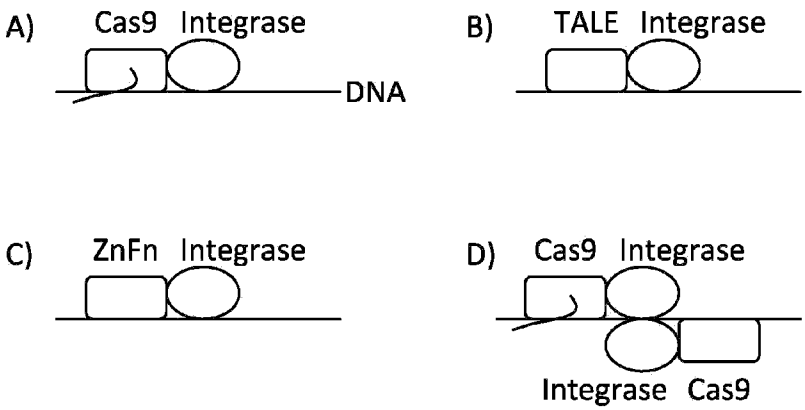

FIG. 1

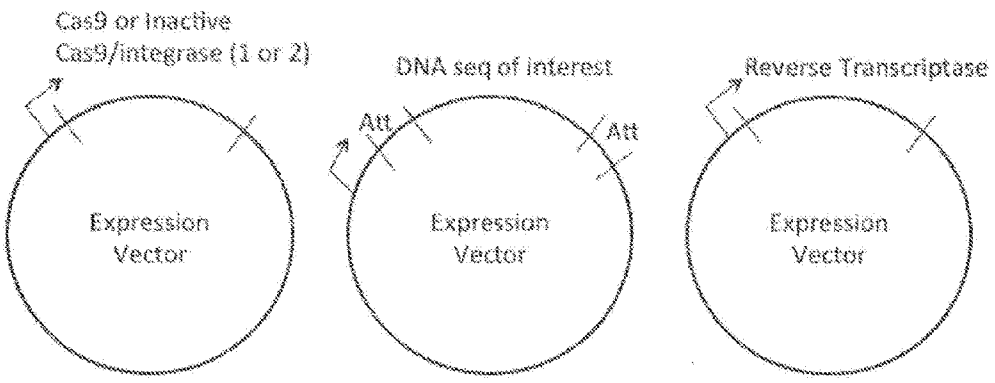
Cas9 or Inactive
Cas9/integrase (1 or 2)
Expression
Vector
DNA seq of interest
Att
Att
Expression
Vector
Reverse Transcriptase
Expression
Vector

FIG. 2

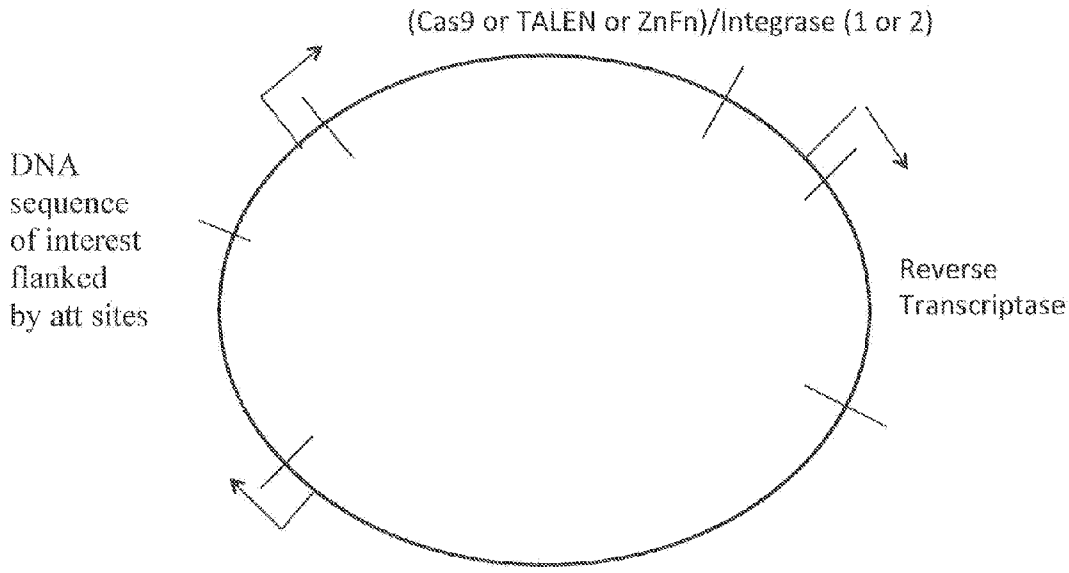
(Cas9 or TALEN or ZnFn)/Integrase (1 or 2)
DNA sequence of interest flanked by att sites
Reverse Transcriptase

FIG. 3

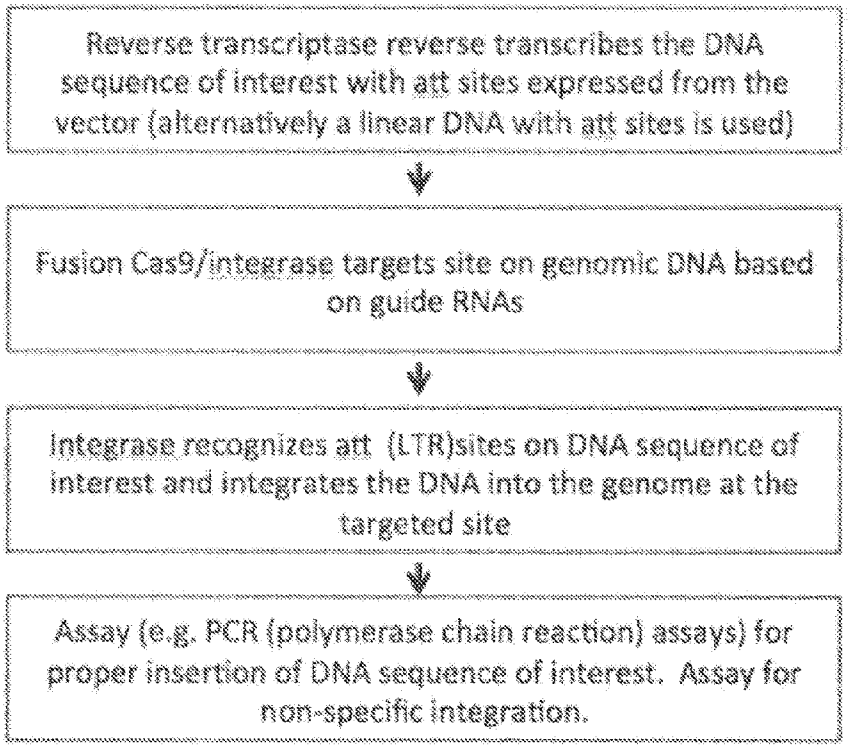
Reverse transcriptase reverse transcribes the DNA sequence of interest with att sites expressed from the vector (alternatively a linear DNA with att sites is used)
Fusion Cas9/integrase targets site on genomic DNA based on guide RNAs
Integrase recognizes att (LTR)sites on DNA sequence of interest and integrates the DNA into the genome at the targeted site
Assay (e.g. PCR (polymerase chain reaction) assays) for proper insertion of DNA sequence of interest. Assay for non-specific integration.

FIG. 4

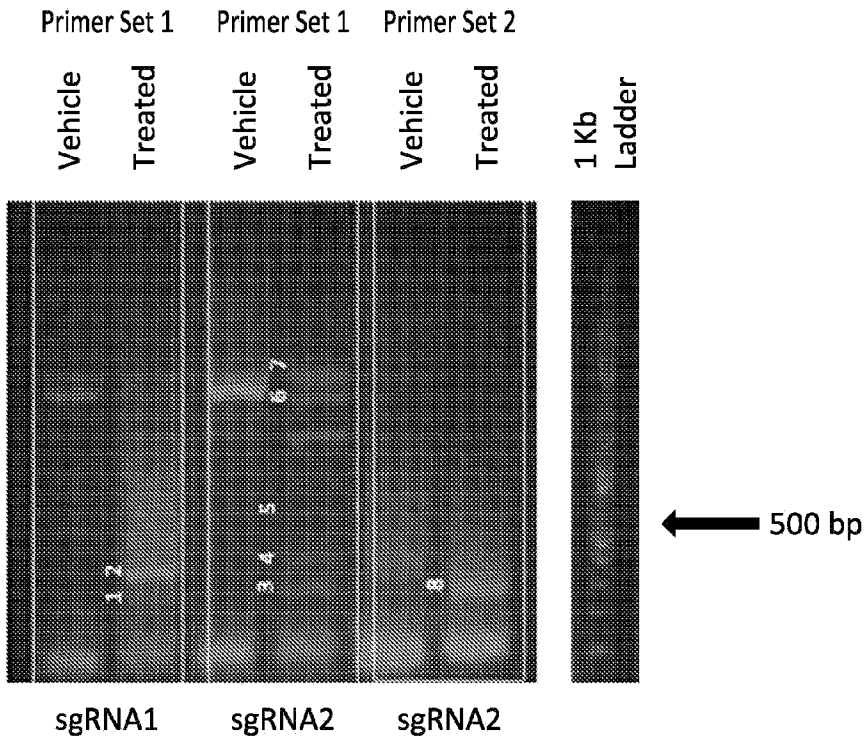
Primer Set 1
Primer Set 1
Primer Set 2
Vehicle
Treated
Vehicle
Treated
Vehicle
Treated
1 Kb Ladder
1 2
3 4 5
6 7
8
500 bp
sgRNA1
sgRNA2
sgRNA2

FIG. 5

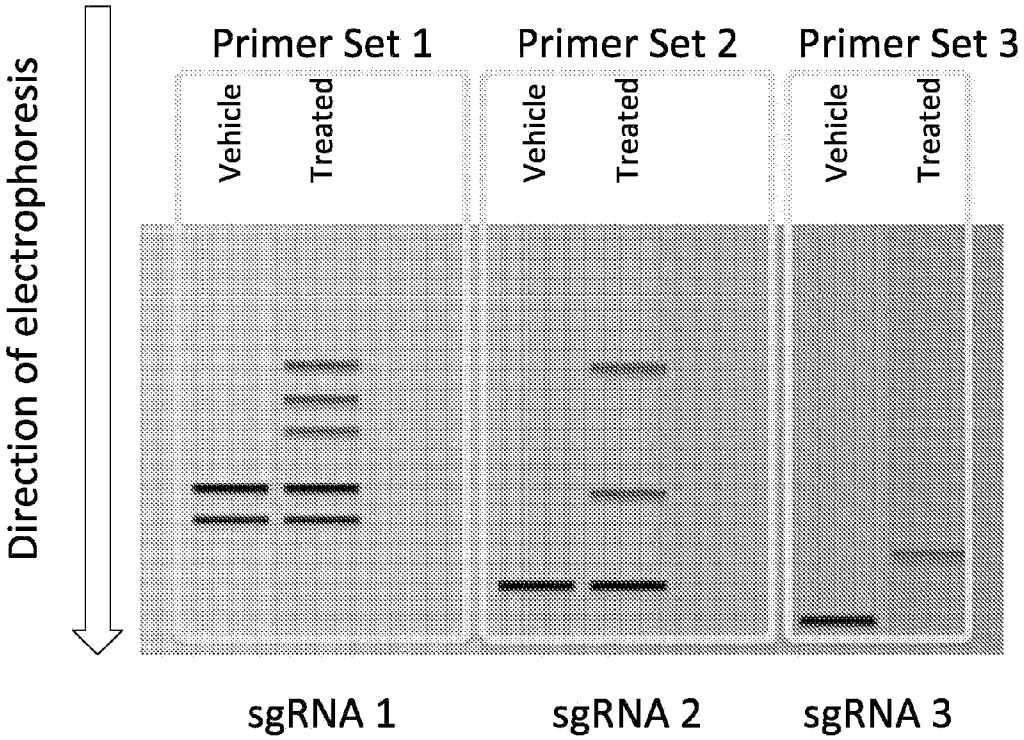
Direction of electrophoresis
Primer Set 1
Primer Set 2
Primer Set 3
Vehicle
Treated
Vehicle
Treated
Vehicle
Treated
sgRNA 1
sgRNA 2
sgRNA 3

FIG. 6

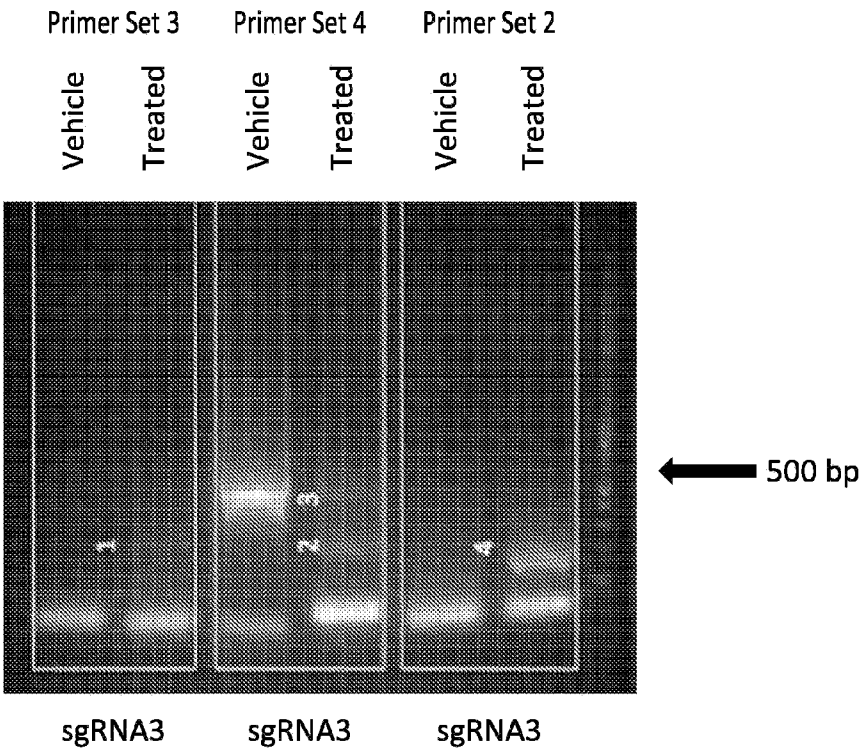
Primer Set 3
Primer Set 4
Primer Set 2
Vehicle
Treated
Vehicle
Treated
Vehicle
Treated
1
3
2
4
500 bp
sgRNA3
sgRNA3
sgRNA3

FIG. 7

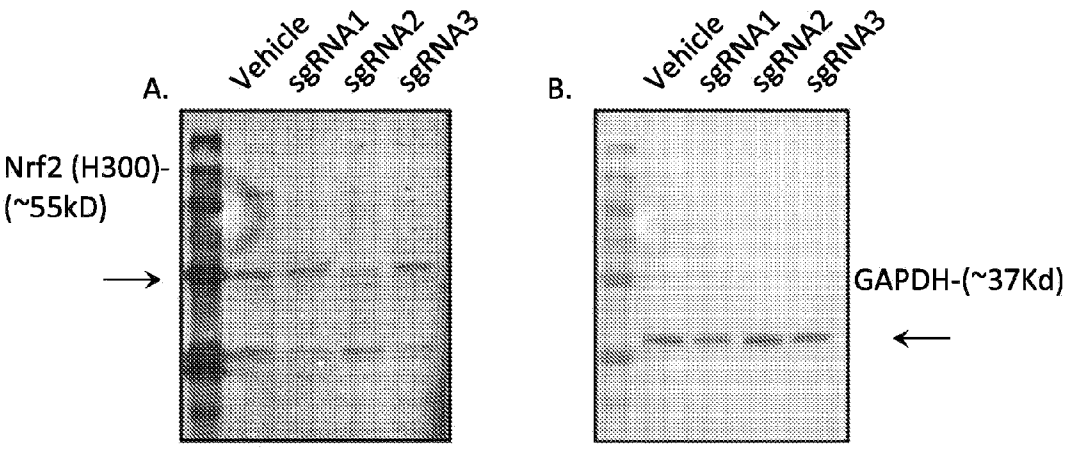
A.
Vehicle
sgRNA1
sgRNA2
sgRNA3
Nrf2 (H300)-
(~55kD)
B.
Vehicle
sgRNA1
sgRNA2
sgRNA3
GAPDH-(~37Kd)

FIG. 8

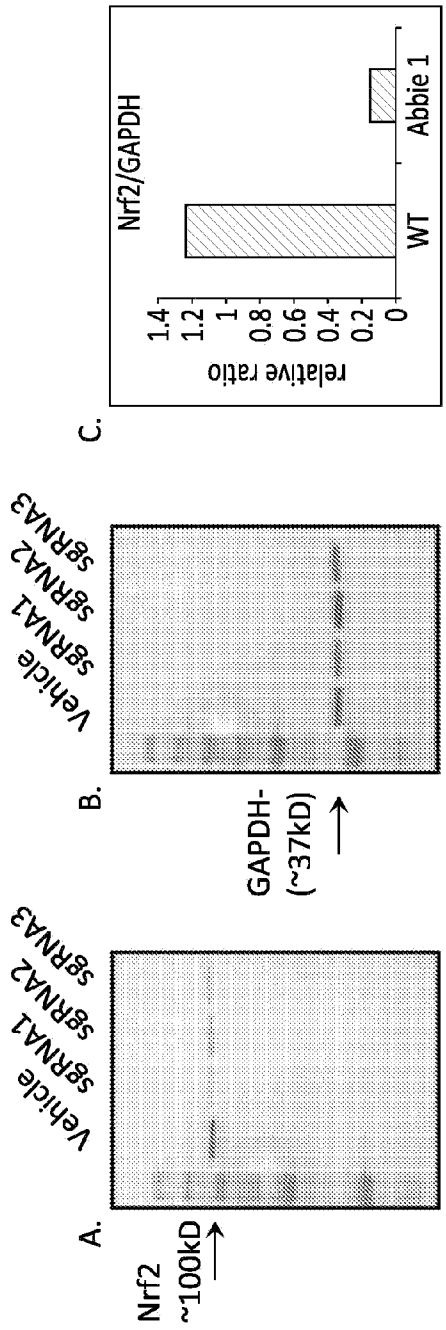
A.
Nrf2
~100kD
Vehicle
sgRNA1
sgRNA2
sgRNA3
B.
GAPDH-
(~37kD)
Vehicle
sgRNA1
sgRNA2
sgRNA3
C.
Nrf2/GAPDH
relative ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
WT
Abbie 1

FIG. 9

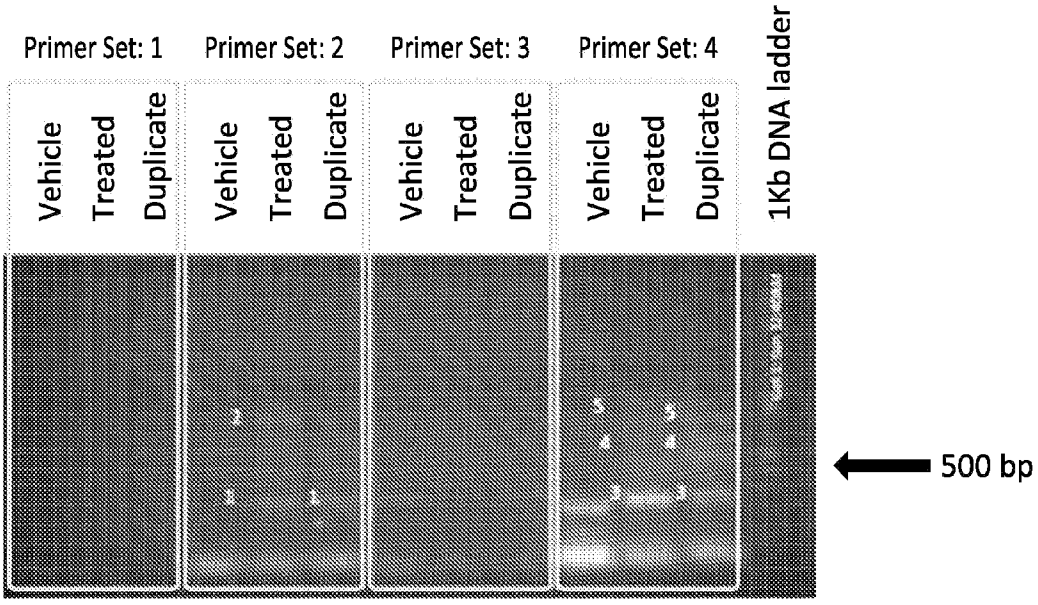
Primer Set: 1
Primer Set: 2
Primer Set: 3
Primer Set: 4
Vehicle
Treated
Duplicate
Vehicle
Treated
Duplicate
Vehicle
Treated
Duplicate
Vehicle
Treated
Duplicate
1Kb DNA ladder
500 bp

FIG. 10

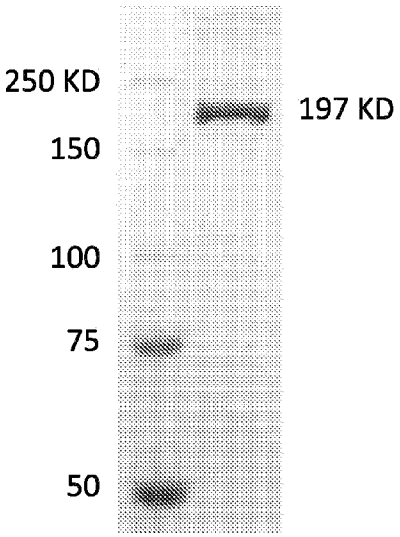

FIG. 11